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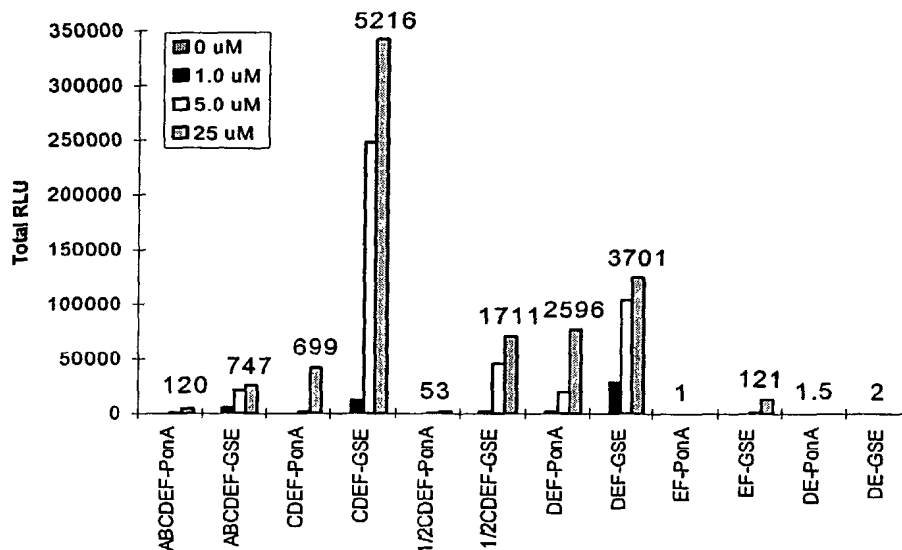
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(54) Title: NOVEL ECDYSONE RECEPTOR/INVERTEBRATE RETINOID X RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM

Analysis of CfEcR Truncations with LmUSPE in 3T3 Cells



(57) Abstract: This invention relates to the field of biotechnology or genetic engineering. Specifically, this invention relates to the field of gene expression. More specifically, this invention relates to a novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system and methods of modulating gene expression in a host cell for applications such as gene therapy, large-scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics and regulation of traits in transgenic organisms.



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NOVEL ECDYSONE RECEPTOR/INVERTEBRATE RETINOID X RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM

FIELD OF THE INVENTION

5 This invention relates to the field of biotechnology or genetic engineering. Specifically, this invention relates to the field of gene expression. More specifically, this invention relates to a novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system and methods of modulating the expression of a gene within a host cell using this inducible gene expression system.

BACKGROUND OF THE INVENTION

10 Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties. However, the citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

15 In the field of genetic engineering, precise control of gene expression is a valuable tool for studying, manipulating, and controlling development and other physiological processes. Gene expression is a complex biological process involving a number of specific protein-protein interactions. In order for gene expression to be triggered, such that it produces the RNA necessary as the first step in protein synthesis, a transcriptional activator must be brought into proximity of a promoter that controls gene transcription. Typically, the transcriptional activator itself is associated with a protein that has at least one DNA binding domain that binds to DNA binding sites present in the promoter regions of genes. Thus, for gene expression to occur, a protein comprising a DNA binding domain and a transactivation domain located at an appropriate distance from the DNA binding domain must be brought into the correct position in the promoter region of the gene.

25 The traditional transgenic approach utilizes a cell-type specific promoter to drive the expression of a designed transgene. A DNA construct containing the transgene is first incorporated into a host genome. When triggered by a transcriptional activator, expression of the transgene occurs in a given cell type.

30 Another means to regulate expression of foreign genes in cells is through inducible promoters. Examples of the use of such inducible promoters include the PR1-a promoter, prokaryotic repressor-operator systems, immunosuppressive-immunophilin systems, and higher eukaryotic transcription activation systems such as steroid hormone receptor systems and are described below.

35 The PR1-a promoter from tobacco is induced during the systemic acquired resistance response following pathogen attack. The use of PR1-a may be limited because it often responds to endogenous materials and external factors such as pathogens, UV-B radiation, and pollutants. Gene regulation systems based on promoters induced by heat shock, interferon and heavy metals have been described

(Wurn et al., 1986, Proc. Natl. Acad. Sci. USA 83: 5414-5418; Arnheiter et al., 1990, Cell 62: 51-61; Filmus et al., 1992, Nucleic Acids Research 20: 27550-27560). However, these systems have limitations due to their effect on expression of non-target genes. These systems are also leaky.

Prokaryotic repressor-operator systems utilize bacterial repressor proteins and the unique operator DNA sequences to which they bind. Both the tetracycline ("Tet") and lactose ("Lac") repressor-operator systems from the bacterium *Escherichia coli* have been used in plants and animals to control gene expression. In the Tet system, tetracycline binds to the TetR repressor protein, resulting in a conformational change that releases the repressor protein from the operator which as a result allows transcription to occur. In the Lac system, a lac operon is activated in response to the presence of lactose, or synthetic analogs such as isopropyl-b-D-thiogalactoside. Unfortunately, the use of such systems is restricted by unstable chemistry of the ligands, *i.e.* tetracycline and lactose, their toxicity, their natural presence, or the relatively high levels required for induction or repression. For similar reasons, utility of such systems in animals is limited.

Immunosuppressive molecules such as FK506, rapamycin and cyclosporine A can bind to immunophilins FKBP12, cyclophilin, *etc.* Using this information, a general strategy has been devised to bring together any two proteins simply by placing FK506 on each of the two proteins or by placing FK506 on one and cyclosporine A on another one. A synthetic homodimer of FK506 (FK1012) or a compound resulted from fusion of FK506-cyclosporine (FKCsA) can then be used to induce dimerization of these molecules (Spencer et al., 1993, *Science* 262 :1019-24; Belshaw et al., 1996, *Proc Natl Acad Sci U S A* 93:4604-7). Gal4 DNA binding domain fused to FKBP12 and VP16 activator domain fused to cyclophilin, and FKCsA compound were used to show heterodimerization and activation of a reporter gene under the control of a promoter containing Gal4 binding sites. Unfortunately, this system includes immunosuppressants that can have unwanted side effects and therefore, limits its use for various mammalian gene switch applications.

Higher eukaryotic transcription activation systems such as steroid hormone receptor systems have also been employed. Steroid hormone receptors are members of the nuclear receptor superfamily and are found in vertebrate and invertebrate cells. Unfortunately, use of steroidal compounds that activate the receptors for the regulation of gene expression, particularly in plants and mammals, is limited due to their involvement in many other natural biological pathways in such organisms. In order to overcome such difficulties, an alternative system has been developed using insect ecdysone receptors (EcR).

Growth, molting, and development in insects are regulated by the ecdysone steroid hormone (molting hormone) and the juvenile hormones (Dhadialla, et al., 1998, *Annu. Rev. Entomol.* 43: 545-569). The molecular target for ecdysone in insects consists of at least ecdysone receptor (EcR) and ultraspiracle protein (USP). EcR is a member of the nuclear steroid receptor super family that is characterized by signature DNA and ligand binding domains, and an activation domain (Koelle et al. 1991, *Cell*, 67: 59-77). EcR receptors are responsive to a number of steroidal compounds such as

ponasterone A and muristerone A. Recently, non-steroidal compounds with ecdysteroid agonist activity have been described, including the commercially available insecticides tebufenozide and methoxyfenozide that are marketed world wide by Rohm and Haas Company (see International Patent Application No. PCT/EP96/00686 and US Patent 5,530,028). Both analogs have exceptional safety
5 profiles to other organisms.

International Patent Applications No. PCT/US97/05330 (WO 97/38117) and PCT/US99/08381 (WO99/58155) disclose methods for modulating the expression of an exogenous gene in which a DNA construct comprising the exogenous gene and an ecdysone response element is activated by a second DNA construct comprising an ecdysone receptor that, in the presence of a ligand therefor, and optionally
10 in the presence of a receptor capable of acting as a silent partner, binds to the ecdysone response element to induce gene expression. The ecdysone receptor of choice was isolated from *Drosophila melanogaster*. Typically, such systems require the presence of the silent partner, preferably retinoid X receptor (RXR), in order to provide optimum activation. In mammalian cells, insect ecdysone receptor (EcR) heterodimerizes with retinoid X receptor (RXR) and regulates expression of target genes in a ligand
15 dependent manner. International Patent Application No. PCT/US98/14215 (WO 99/02683) discloses that the ecdysone receptor isolated from the silk moth *Bombyx mori* is functional in mammalian systems without the need for an exogenous dimer partner.

U.S. Patent No. 5,880,333 discloses a *Drosophila melanogaster* EcR and ultraspiracle (USP) heterodimer system used in plants in which the transactivation domain and the DNA binding domain are
20 positioned on two different hybrid proteins. Unfortunately, this system is not effective for inducing reporter gene expression in animal cells (for comparison, see Example 1.2, below).

In each of these cases, the transactivation domain and the DNA binding domain (either as native EcR as in International Patent Application No. PCT/US98/14215 or as modified EcR as in International Patent Application No. PCT/US97/05330) were incorporated into a single molecule and the other
25 heterodimeric partners, either USP or RXR, were used in their native state.

Drawbacks of the above described EcR-based gene regulation systems include a considerable background activity in the absence of ligands and non-applicability of these systems for use in both plants and animals (see U.S. Patent No. 5,880,333). For most applications that rely on modulating gene expression, these EcR-based systems are undesirable. Therefore, a need exists in the art for improved
30 systems to precisely modulate the expression of exogenous genes in both plants and animals. Such improved systems would be useful for applications such as gene therapy, large-scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics and regulation of traits in transgenic animals. Improved systems that are simple, compact, and dependent on ligands that are relatively inexpensive, readily available, and of low toxicity to the host would prove useful for
35 regulating biological systems.

Recently, Applicants have shown that an ecdysone receptor-based inducible gene expression system in which the transactivation and DNA binding domains are separated from each other by placing

them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT/US01/09050, incorporated herein in its entirety by reference). This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems
5 disclosed in applications PCT/US97/05330 and PCT/US98/14215.

Applicants previously demonstrated that an ecdysone receptor-based gene expression system in partnership with a dipteran (*Drosophila melanogaster*) or a lepidopteran (*Choristoneura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor-based gene expression system in partnership with a vertebrate retinoid X receptor (RXR) is inducible in
10 mammalian cells (pending application PCT/US01/09050). Applicants have now made the surprising discovery that a non-dipteran and non-lepidopteran invertebrate RXR homolog can function similar to vertebrate RXR in an ecdysone receptor-based inducible gene expression system. As described herein, Applicants' novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system provides an improved inducible gene expression system in yeast and mammalian cells that is
15 characterized by increased ligand sensitivity and magnitude of transactivation.

SUMMARY OF THE INVENTION

The present invention relates to a novel ecdysone receptor/invertebrate retinoid X receptor-based
20 inducible gene expression system, novel receptor polynucleotides and polypeptides for use in the novel inducible gene expression system, and methods of modulating the expression of a gene within a host cell using this inducible gene expression system. In particular, Applicants' invention relates to an improved gene expression modulation system comprising a polynucleotide encoding a ligand binding domain of an invertebrate retinoid X receptor (RXR) polypeptide.

Specifically, the present invention relates to a gene expression modulation system comprising: a)
25 a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of
30 being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) an invertebrate retinoid X receptor ligand binding domain.

The present invention also relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide
35 that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an invertebrate retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being

expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

The present invention also relates to a gene expression modulation system according to the invention further comprising c) a third gene expression cassette comprising: i) a response element to
5 which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated.

The present invention also relates to a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid
10 polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

The present invention also relates to an isolated polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated
15 with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

The present invention also relates to an isolated polynucleotide encoding a truncated invertebrate RXR polypeptide, wherein the truncation mutation affects ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide.

20 The present invention also relates to an isolated polynucleotide encoding a truncated invertebrate RXR polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated invertebrate RXR polypeptide and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

The present invention also relates to an isolated polypeptide encoded by a polynucleotide
25 according to Applicants' invention.

The present invention also relates to an isolated hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

30 The present invention relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation, wherein the invertebrate RXR polypeptide is encoded by a polynucleotide according to the invention.

Thus, the present invention also relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity of said
35 invertebrate RXR polypeptide.

The present invention also relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the

truncated invertebrate RXR polypeptide and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain to which the DNA binding domain from the first hybrid polypeptide of the gene expression modulation system binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide of the gene expression modulation system; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

Applicants' invention also provides a method of modulating the expression of a gene in a host cell comprising a gene expression cassette comprising a response element comprising a domain to which the DNA binding domain from the first hybrid polypeptide of the gene expression modulation system binds; a promoter that is activated by the transactivation domain of the second hybrid polypeptide of the gene expression modulation system; and a gene whose expression is to be modulated; wherein the method comprises the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host, expression of the gene is modulated.

Applicants' invention also provides an isolated host cell comprising an inducible gene expression system according to the invention. The present invention also relates to an isolated host cell comprising a gene expression cassette, a polynucleotide, or a polypeptide according to the invention. Accordingly, Applicants' invention also relates to a non-human organism comprising a host cell according to the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Transactivation of reporter genes through VP16MmRXRDEF, VP16MmRXREF, VP16LmUSP, and VP16CfUSP constructs transfected into NIH3T3 cells along with GAL4CfEcRCDEF, pFRLuc and pTKRL plasmid DNAs by a non-steroidal ligand.

Figure 2: Transactivation of reporter genes through VP16MmRXRDEF, VP16MmRXREF, VP16LmUSP, and VP16CfUSP constructs transfected into NIH3T3 cells along with GAL4CfEcRDEF, pFRLuc and pTKRL plasmid DNAs by a non-steroidal ligand.

Figure 3: Amino acid sequence alignments of the EF domains of six vertebrate RXRs (A) and six invertebrate RXRs (B). Helices 1-12 are denoted as H1-H12 and β pleated sheets are denoted as S1 and S2. F denotes the F domain junction.

Figure 4: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GAL4CfEcR1/2CDEF, GAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16MmRXRDEF, pFRLuc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

- 5 Figure 5: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GAL4CfEcR1/2CDEF, GAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16MmRXREF, pFRLuc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

- Figure 6: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, 10 GAL4CfEcR1/2CDEF, GAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16LmUSPDEF, pFRLuc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

- Figure 7: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, 15 GAL4CfEcR1/2CDEF, GAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16LmUSPEF, pFRLuc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 8: Expression data of various truncated MmRXR/LmUSP receptor constructs transfected into NIH3T3 cells along with GAL4CfEcRDEF, pFRLuc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

- 20 Figure 9: Expression data of CfUSP-EF, DmUSP-EF, LmUSP-EF, MmRXR α -EF, AmaRXR1-EF and AmaRXR2-EF ligand binding domains fused to VP16 along with GAL4/CfEcR-DEF and pFRLuc in NIH3T3 cells in the presence of non-steroidal (GSE) ligand or PonA ligand.

Figure 10: Expression data of GAL4:CfEcR-DEF/VP16:LmUSP-EF in stably transfected CHO cells comprising a reporter plasmid pFRLuc in the presence of non-steroidal ligand or PonA ligand.

- 25 Figure 11: Expression data of a LexA:CfEcR-CDEF receptor construct transfected into NIH3T3 cells along with 8XLexAopFRLuc and VP16:CfUSP-EF, VP16:LmUSP-EF, VP16:MmRXR α -EF or VP16:DmUSP-EF in the presence of non-steroidal ligand or PonA ligand.

- Figure 12: NIH3T3 cells were transfected with different combinations of GAL4:CfEcR-CDEF or LexA:CfEcR-CDEF, 8XLexAopFRLuc and VP16:LmUSP-EF or B42:LmUSP-EF in the presence of 30 non-steroidal ligand.

Figure 13: Effect of 9-cis-retinoic acid on transactivation potential of the GAL4CfEcR-DEF/VP16LmUSP-EF gene switch along with pFRLuc in NIH 3T3 cells in the presence of non-steroid (GSE) and 9-cis-retinoic acid (9Cis) for 48 hours.

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DETAILED DESCRIPTION OF THE INVENTION

Applicants have developed a novel ecdysone receptor-based inducible gene expression system

comprising an invertebrate retinoid X receptor polypeptide. Applicants have also shown that truncations of an invertebrate RXR polypeptide are also functional within this gene expression system and that these mutational effects may increase or reduce ligand binding activity or ligand sensitivity and may be steroid or non-steroid specific. Thus, Applicants' invention provides an ecdysone receptor/invertebrate RXR-
5 based inducible gene expression system useful for modulating expression of a gene of interest in a host cell. In a particularly desirable embodiment, Applicants' invention provides an inducible gene expression system that has a reduced level of background gene expression and responds to submicromolar concentrations of non-steroidal ligand. Thus, Applicants' novel inducible gene expression system and its use in methods of modulating gene expression in a host cell overcome the
10 limitations of currently available inducible expression systems and provide the skilled artisan with an effective means to control gene expression.

The present invention is useful for applications such as gene therapy, large scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics, proteomics, metabolomics, and regulation of traits in transgenic organisms, where control of gene expression levels is
15 desirable. An advantage of Applicants' invention is that it provides a means to regulate gene expression and to tailor expression levels to suit the user's requirements.

DEFINITIONS

In this disclosure, a number of terms and abbreviations are used. The following definitions are
20 provided and should be helpful in understanding the scope and practice of the present invention.

In a specific embodiment, the term "about" or "approximately" means within 20%, preferably within 10%, more preferably within 5%, and even more preferably within 1% of a given value or range.

The term "substantially free" means that a composition comprising "A" (where "A" is a single protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B"
25 comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A + B species in the composition, most preferably at least about 99% by weight. It is also preferred that a composition, which is substantially free of contamination, contain only a single molecular weight species having the
30 activity or characteristic of the species of interest.

The term "isolated" for the purposes of the present invention designates a biological material (nucleic acid or protein) that has been removed from its original environment (the environment in which it is naturally present). For example, a polynucleotide present in the natural state in a plant or an animal is not isolated, however the same polynucleotide separated from the adjacent nucleic acids in which it is
35 naturally present, is considered "isolated". The term "purified" does not require the material to be present in a form exhibiting absolute purity, exclusive of the presence of other compounds. It is rather a relative definition.

A polynucleotide is in the "purified" state after purification of the starting material or of the natural material by at least one order of magnitude, preferably 2 or 3 and preferably 4 or 5 orders of magnitude.

A "nucleic acid" is a polymeric compound comprised of covalently linked subunits called
 5 nucleotides. Nucleic acid includes polyribonucleic acid (RNA) and polydeoxyribonucleic acid (DNA), both of which may be single-stranded or double-stranded. DNA includes but is not limited to cDNA, genomic DNA, plasmids DNA, synthetic DNA, and semi-synthetic DNA. DNA may be linear, circular, or supercoiled.

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides
 10 (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary
 15 structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence
 20 homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

The term "fragment" will be understood to mean a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be,
 25 where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least 6, 8, 9, 10, 12, 15, 18, 20, 21, 22, 23, 24, 25, 30, 39, 40, 42, 45, 48, 50, 51, 54, 57, 60, 63, 66, 70, 75, 78, 80, 90, 100, 105, 120, 135, 150, 200, 300, 500, 720, 900, 1000 or 1500 consecutive nucleotides of a nucleic acid according to the invention.

30 As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

A "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and
 35 genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in

nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and/or coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. A chimeric gene may comprise coding sequences derived from different sources and/or regulatory sequences derived from different sources. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

"Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

The term "genome" includes chromosomal as well as mitochondrial, chloroplast and viral DNA or RNA.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook *et al.*, 1989 *infra*). Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , *e.g.*, 40% formamide, with 5x or 6x SCC. High stringency hybridization conditions correspond to the highest T_m , *e.g.*, 50% formamide, 5x or 6x SCC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible.

The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine. Accordingly, the instant invention also includes isolated nucleic acid fragments that are complementary to the complete sequences as disclosed or used herein as well as those substantially similar nucleic acid sequences.

In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C.

Post-hybridization washes also determine stringency conditions. One set of preferred conditions
 5 uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 minutes (min), then repeated with 2X SSC, 0.5% SDS at 45°C for 30 minutes, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 minutes. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses
 10 two final washes in 0.1X SSC, 0.1% SDS at 65°C. Hybridization requires that the two nucleic acids comprise complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible.

The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of
 15 similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook *et al.*, *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of
 20 mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook *et al.*, *supra*, 11.7-11.8).

In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides.
 25 Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

The term "probe" refers to a single-stranded nucleic acid molecule that can base pair with a complementary single stranded target nucleic acid to form a double-stranded molecule. As used herein, the term "oligonucleotide" refers to a nucleic acid, generally of at least 18 nucleotides, that is
 30 hybridizable to a genomic DNA molecule, a cDNA molecule, a plasmid DNA or an mRNA molecule. Oligonucleotides can be labeled, *e.g.*, with ^{32}P -nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. A labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. Oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of a nucleic acid, or to detect the presence of a nucleic acid.
 35 An oligonucleotide can also be used to form a triple helix with a DNA molecule. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer. Accordingly, oligonucleotides can be prepared with non-naturally occurring phosphoester analog bonds, such as

thioester bonds, etc.

A “primer” is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

5 “Polymerase chain reaction” is abbreviated PCR and means an *in vitro* method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the
10 presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

“Reverse transcription-polymerase chain reaction” is abbreviated RT-PCR and means an *in vitro* method for enzymatically producing a target cDNA molecule or molecules from an RNA molecule or molecules, followed by enzymatic amplification of a specific nucleic acid sequence or sequences within
15 the target cDNA molecule or molecules as described above. RT-PCR also provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

A DNA “coding sequence” is a double-stranded DNA sequence that is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory
20 sequences. “Suitable regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure. The
25 boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from mRNA, genomic DNA sequences, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

30 “Open reading frame” is abbreviated ORF and means a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

The term “head-to-head” is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-head orientation
35 when the 5' end of the coding strand of one polynucleotide is adjacent to the 5' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds away from the 5' end of the other polynucleotide. The term “head-to-head” may be abbreviated (5')-to-(5')

and may also be indicated by the symbols ($\leftarrow \rightarrow$) or ($3' \leftarrow 5' 5' \rightarrow 3'$).

The term "tail-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a tail-to-tail orientation when the 3' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other
 5 polynucleotide, whereby the direction of transcription of each polynucleotide proceeds toward the other polynucleotide. The term "tail-to-tail" may be abbreviated (3')-to-(3') and may also be indicated by the symbols ($\rightarrow \leftarrow$) or ($5' \rightarrow 3' 3' \leftarrow 5'$).

The term "head-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-tail orientation
 10 when the 5' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds in the same direction as that of the other polynucleotide. The term "head-to-tail" may be abbreviated (5')-to-(3') and may also be indicated by the symbols ($\rightarrow \rightarrow$) or ($5' \rightarrow 3' 5' \rightarrow 3'$).

The term "downstream" refers to a nucleotide sequence that is located 3' to reference nucleotide
 15 sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

The term "upstream" refers to a nucleotide sequence that is located 5' to reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on
 20 the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

The terms "restriction endonuclease" and "restriction enzyme" refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

"Homologous recombination" refers to the insertion of a foreign DNA sequence into another
 25 DNA molecule, e.g., insertion of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous
 30 recombination.

Several methods known in the art may be used to propagate a polynucleotide according to the invention. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As described herein, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal
 35 viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

A "vector" is any means for the cloning of and/or transfer of a nucleic acid into a host cell. A

vector may be a replicon to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral means for

5 introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes, etc. Possible vectors include, for example, plasmids or modified viruses including, for example bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives, or the Bluescript vector. For example, the insertion of the DNA fragments corresponding to response

10 elements and promoters into a suitable vector can be accomplished by ligating the appropriate DNA fragments into a chosen vector that has complementary cohesive termini. Alternatively, the ends of the DNA molecules may be enzymatically modified or any site may be produced by ligating nucleotide sequences (linkers) into the DNA termini. Such vectors may be engineered to contain selectable marker genes that provide for the selection of cells that have incorporated the marker into the cellular genome.

15 Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker.

Viral vectors, and particularly retroviral vectors, have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Viral vectors that can be used include but are not limited to retrovirus, adeno-associated virus, pox, baculovirus, vaccinia, herpes simplex,

20 Epstein-Barr, adenovirus, geminivirus, and caulimovirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers. In addition to a nucleic acid, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (transfer to which tissues, duration of expression, etc.).

25 The term "plasmid" refers to an extra chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or

30 recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

A "cloning vector" is a "replicon", which is a unit length of a nucleic acid, preferably DNA, that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the

35 attached segment. Cloning vectors may be capable of replication in one cell type and expression in another ("shuttle vector").

Vectors may be introduced into the desired host cells by methods known in the art, *e.g.*,

transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, e.g., Wu et al., 1992, J. Biol. Chem. 267: 963-967; Wu and Wu, 1988, J. Biol. Chem. 263: 14621-14624; and Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

5 A polynucleotide according to the invention can also be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome-mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., 1987, PNAS 84: 7413; Mackey, et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85: 8027-8031; and
10 Ulmer et al., 1993, Science 259: 1745-1748). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, 1989, Science 337: 387-388). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO95/18863 and WO96/17823, and in U.S. Patent No. 5,459,127. The use of lipofection to introduce exogenous genes into the specific organs *in*
15 *vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly preferred in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting (Mackey, et al., 1988, *supra*). Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to
20 liposomes chemically.

Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (e.g., WO95/21931), peptides derived from DNA binding proteins (e.g., WO96/25508), or a cationic polymer (e.g., WO95/21931).

It is also possible to introduce a vector *in vivo* as a naked DNA plasmid (see U.S. Patents
25 5,693,622, 5,589,466 and 5,580,859). Receptor-mediated DNA delivery approaches can also be used (Curiel et al., 1992, Hum. Gene Ther. 3: 147-154; and Wu and Wu, 1987, J. Biol. Chem. 262: 4429-4432).

The term "transfection" means the uptake of exogenous or heterologous RNA or DNA by a cell. A cell has been "transfected" by exogenous or heterologous RNA or DNA when such RNA or DNA has
30 been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous RNA or DNA when the transfected RNA or DNA effects a phenotypic change. The transforming RNA or DNA can be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic
35 acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

The term "genetic region" will refer to a region of a nucleic acid molecule or a nucleotide

sequence that comprises a gene encoding a polypeptide.

In addition, the recombinant vector comprising a polynucleotide according to the invention may include one or more origins for replication in the cellular hosts in which their amplification or their expression is sought, markers or selectable markers.

5 The term “selectable marker” means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene’s effect, *i.e.*, resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and
10 used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like.

 The term “reporter gene” means a nucleic acid encoding an identifying factor that is able to be identified based upon the reporter gene’s effect, wherein the effect is used to track the inheritance of a
15 nucleic acid of interest, to identify a cell or organism that has inherited the nucleic acid of interest, and/or to measure gene expression induction or transcription. Examples of reporter genes known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), β -galactosidase (LacZ), β -glucuronidase (Gus), and the like. Selectable marker genes may also be considered reporter genes.

20 “Promoter” refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or
25 cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as “cell-specific promoters” or “tissue-specific promoters”. Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are
30 commonly referred to as “developmentally-specific promoters” or “cell differentiation-specific promoters”. Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as “inducible promoters” or “regulatable promoters”. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been
35 completely defined, DNA fragments of different lengths may have identical promoter activity.

 A “promoter sequence” is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the

present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well
 5 as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced (if the coding sequence contains introns) and translated into the protein encoded by the coding sequence.

1 0 "Transcriptional and translational control sequences" are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

The term "response element" means one or more cis-acting DNA elements which confer responsiveness on a promoter mediated through interaction with the DNA-binding domains of the first
 1 5 chimeric gene. This DNA element may be either palindromic (perfect or imperfect) in its sequence or composed of sequence motifs or half sites separated by a variable number of nucleotides. The half sites can be similar or identical and arranged as either direct or inverted repeats or as a single half site or multimers of adjacent half sites in tandem. The response element may comprise a minimal promoter isolated from different organisms depending upon the nature of the cell or organism into which the
 2 0 response element will be incorporated. The DNA binding domain of the first hybrid protein binds, in the presence or absence of a ligand, to the DNA sequence of a response element to initiate or suppress transcription of downstream gene(s) under the regulation of this response element. Examples of DNA sequences for response elements of the natural ecdysone receptor include: RRGG/TTCANTGAC/ACY (see Cherbas L., et. al., (1991), *Genes Dev.* 5: 120-131); AGGTCAN_(n)AGGTCA, where N_(n) can be one
 2 5 or more spacer nucleotides (see D'Avino PP., et. al., (1995), *Mol. Cell. Endocrinol* 113: 1-9); and GGGTTGAATGAATTT (see Antoniewski C., et. al., (1994), *Mol. Cell Biol.* 14: 4465-4474).

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably
 3 0 linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

3 5 The terms "cassette", "expression cassette" and "gene expression cassette" refer to a segment of DNA that can be inserted into a nucleic acid or polynucleotide at specific restriction sites or by homologous recombination. The segment of DNA comprises a polynucleotide that encodes a

polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation. "Transformation cassette" refers to a specific vector comprising a polynucleotide that encodes a polypeptide of interest and having elements in addition to the polynucleotide that facilitate transformation of a particular host cell. Cassettes,

5 expression cassettes, gene expression cassettes and transformation cassettes of the invention may also comprise elements that allow for enhanced expression of a polynucleotide encoding a polypeptide of interest in a host cell. These elements may include, but are not limited to: a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

1.0 For purposes of this invention, the term "gene switch" refers to the combination of a response element associated with a promoter, and an EcR based system which, in the presence of one or more ligands, modulates the expression of a gene into which the response element and promoter are incorporated.

The terms "modulate" and "modulates" mean to induce, reduce or inhibit nucleic acid or gene
1.5 expression, resulting in the respective induction, reduction or inhibition of protein or polypeptide production.

The plasmids or vectors according to the invention may further comprise at least one promoter suitable for driving expression of a gene in a host cell. The term "expression vector" means a vector, plasmid or vehicle designed to enable the expression of an inserted nucleic acid sequence following
2.0 transformation into the host. The cloned gene, i.e., the inserted nucleic acid sequence, is usually placed under the control of control elements such as a promoter, a minimal promoter, an enhancer, or the like. Initiation control regions or promoters, which are useful to drive expression of a nucleic acid in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to: viral promoters,
2.5 bacterial promoters, animal promoters, mammalian promoters, synthetic promoters, constitutive promoters, tissue specific promoter, developmental specific promoters, inducible promoters, light regulated promoters; *CYC1*, *HIS3*, *GAL1*, *GAL4*, *GAL10*, *ADH1*, *PGK*, *PHO5*, *GAPDH*, *ADC1*, *TRP1*, *URA3*, *LEU2*, *ENO*, *TPI*, alkaline phosphatase promoters (useful for expression in *Saccharomyces*); *AOX1* promoter (useful for expression in *Pichia*); β -lactamase, *lac*, *ara*, *tet*, *trp*, *lP_L*, *lP_R*, *T7*, *tac*, and
3.0 *trc* promoters (useful for expression in *Escherichia coli*); light regulated-promoters; animal and mammalian promoters known in the art include, but are not limited to, the SV40 early (SV40e) promoter region, the promoter contained in the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV), the promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), the cytomegalovirus (CMV) early promoter, the herpes simplex virus (HSV) thymidine kinase (TK) promoter, an elongation
3.5 factor 1 alpha (EF1) promoter, a phosphoglycerate kinase (PGK) promoter, a ubiquitin (Ubc) promoter, an albumin promoter, the regulatory sequences of the mouse metallothionein-L promoter and transcriptional control regions, the ubiquitous promoters (HPRT, vimentin, α -actin, tubulin and the like),

the promoters of the intermediate filaments (desmin, neurofilaments, keratin, GFAP, and the like), the promoters of therapeutic genes (of the MDR, CFTR or factor VIII type, and the like), pathogenesis or disease related-promoters, and promoters that exhibit tissue specificity and have been utilized in transgenic animals, such as the elastase I gene control region which is active in pancreatic acinar cells; 5 insulin gene control region active in pancreatic beta cells, immunoglobulin gene control region active in lymphoid cells, mouse mammary tumor virus control region active in testicular, breast, lymphoid and mast cells; albumin gene, Apo AI and Apo AII control regions active in liver, alpha-fetoprotein gene control region active in liver, alpha 1-antitrypsin gene control region active in the liver, beta-globin gene control region active in myeloid cells, myelin basic protein gene control region active in oligodendrocyte 1.0 cells in the brain, myosin light chain-2 gene control region active in skeletal muscle, and gonadotropic releasing hormone gene control region active in the hypothalamus, pyruvate kinase promoter, villin promoter, promoter of the fatty acid binding intestinal protein, promoter of the smooth muscle cell α -actin, and the like. In addition, these expression sequences may be modified by addition of enhancer or regulatory sequences and the like.

1.5 Enhancers that may be used in embodiments of the invention include but are not limited to: an SV40 enhancer, a cytomegalovirus (CMV) enhancer, an elongation factor 1 (EF1) enhancer, yeast enhancers, viral gene enhancers, and the like.

Termination control regions, *i.e.*, terminator or polyadenylation sequences, may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, 2.0 however, it is most preferred if included. In a preferred embodiment of the invention, the termination control region may be comprise or be derived from a synthetic sequence, synthetic polyadenylation signal, an SV40 late polyadenylation signal, an SV40 polyadenylation signal, a bovine growth hormone (BGH) polyadenylation signal, viral terminator sequences, or the like.

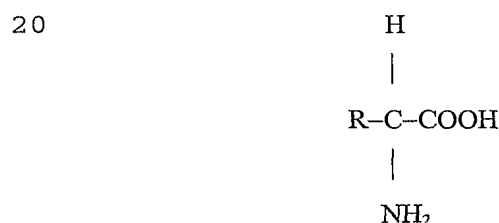
The terms "3' non-coding sequences" or "3' untranslated region (UTR)" refer to DNA sequences 2.5 located downstream (3') of a coding sequence and may comprise polyadenylation [poly(A)] recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

"Regulatory region" means a nucleic acid sequence which regulates the expression of a second 3.0 nucleic acid sequence. A regulatory region may include sequences which are naturally responsible for expressing a particular nucleic acid (a homologous region) or may include sequences of a different origin that are responsible for expressing different proteins or even synthetic proteins (a heterologous region). In particular, the sequences can be sequences of prokaryotic, eukaryotic, or viral genes or derived sequences that stimulate or repress transcription of a gene in a specific or non-specific manner and in an 3.5 inducible or non-inducible manner. Regulatory regions include origins of replication, RNA splice sites, promoters, enhancers, transcriptional termination sequences, and signal sequences which direct the polypeptide into the secretory pathways of the target cell.

A regulatory region from a "heterologous source" is a regulatory region that is not naturally associated with the expressed nucleic acid. Included among the heterologous regulatory regions are regulatory regions from a different species, regulatory regions from a different gene, hybrid regulatory sequences, and regulatory sequences which do not occur in nature, but which are designed by one having
5 ordinary skill in the art.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)"
10 refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene. The complementarity of an
15 antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

A "polypeptide" is a polymeric compound comprised of covalently linked amino acid residues. Amino acids have the following general structure:



25 Amino acids are classified into seven groups on the basis of the side chain R: (1) aliphatic side chains, (2) side chains containing a hydroxylic (OH) group, (3) side chains containing sulfur atoms, (4) side chains containing an acidic or amide group, (5) side chains containing a basic group, (6) side chains containing an aromatic ring, and (7) proline, an imino acid in which the side chain is fused to the amino group. A polypeptide of the invention preferably comprises at least about 14 amino acids.

30 A "protein" is a polypeptide that performs a structural or functional role in a living cell.

An "isolated polypeptide" or "isolated protein" is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids). "Isolated" is not meant to exclude artificial or synthetic mixtures with other compounds, or the presence of impurities which do not interfere with
35 biological activity, and which may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into a pharmaceutically acceptable preparation.

"Fragment" of a polypeptide according to the invention will be understood to mean a polypeptide

whose amino acid sequence is shorter than that of the reference polypeptide and which comprises, over the entire portion with these reference polypeptides, an identical amino acid sequence. Such fragments may, where appropriate, be included in a larger polypeptide of which they are a part. Such fragments of a polypeptide according to the invention may have a length of at least 2, 3, 4, 5, 6, 8, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 100, 200, 240, or 300 amino acids.

A "variant" of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements. These variants may include, *inter alia*: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to the polypeptide or protein, (c) variants in which one or more of the amino acids includes a substituent group, and (d) variants in which the polypeptide or protein is fused with another polypeptide such as serum albumin. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques, are known to persons having ordinary skill in the art. A variant polypeptide preferably comprises at least about 14 amino acids.

A "heterologous protein" refers to a protein not naturally produced in the cell.

A "mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

The term "signal peptide" refers to an amino terminal polypeptide preceding the secreted mature protein. The signal peptide is cleaved from and is therefore not present in the mature protein. Signal peptides have the function of directing and translocating secreted proteins across cell membranes. Signal peptide is also referred to as signal protein.

A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms.

The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct

comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the
5 digested fragments.

As used herein, the term "homologous" in all its grammatical forms and spelling variations refers to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (*e.g.*, the immunoglobulin superfamily) and homologous proteins from different species (*e.g.*, myosin light chain, etc.) (Reeck et al., 1987, Cell 50: 667.). Such proteins (and their
10 encoding genes) have sequence homology, as reflected by their high degree of sequence similarity. However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that may or may not
15 share a common evolutionary origin (*see* Reeck et al., 1987, Cell 50:667).

In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially similar" when at least about 50% (preferably at least about 75%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software
20 available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, *e.g.*, Sambrook *et al.*, 1989, *supra*.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the
25 functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially
30 affect the functional properties of the resulting transcript. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this
35 invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS), with the sequences exemplified herein. Substantially similar nucleic acid fragments of the instant invention are those

nucleic acid fragments whose DNA sequences are at least 70% identical to the DNA sequence of the nucleic acid fragments reported herein. Preferred substantially nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 80% identical to the DNA sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are at least 90% identical to the DNA sequence of the nucleic acid fragments reported herein. Even more preferred are nucleic acid fragments that are at least 95% identical to the DNA sequence of the nucleic acid fragments reported herein.

Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than about 40% of the amino acids are identical, or greater than 60% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*, Madison, Wisconsin) pileup program.

The term "corresponding to" is used herein to refer to similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. A nucleic acid or amino acid sequence alignment may include spaces. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215: 403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence.

The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University

Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences may be performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method may be selected: KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software will include but is not limited to the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), BLASTP, BLASTN, BLASTX (Altschul et al., *J. Mol. Biol.* 215: 403-410 (1990), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

"Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments that are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well-established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

35

GENE EXPRESSION MODULATION SYSTEM OF THE INVENTION

Applicants have previously shown that separating the transactivation and DNA binding domains

by placing them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT/US01/09050). This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in International Patent

- 5 Applications PCT/US97/05330 and PCT/US98/14215. The two-hybrid system exploits the ability of a pair of interacting proteins to bring the transcription activation domain into a more favorable position relative to the DNA binding domain such that when the DNA binding domain binds to the DNA binding site on the gene, the transactivation domain more effectively activates the promoter (see, for example, U.S. Patent No. 5,283,173). Briefly, the two-hybrid gene expression system comprises two gene
- 10 expression cassettes; the first encoding a DNA binding domain fused to a nuclear receptor polypeptide, and the second encoding a transactivation domain fused to a different nuclear receptor polypeptide. In the presence of ligand, the interaction of the first polypeptide with the second polypeptide effectively tethers the DNA binding domain to the transactivation domain. Since the DNA binding and transactivation domains reside on two different molecules, the background activity in the absence of
- 15 ligand is greatly reduced.

- The two-hybrid ecdysone receptor-based gene expression modulation system may be either heterodimeric and homodimeric. A functional EcR complex generally refers to a heterodimeric protein complex consisting of two members of the steroid receptor family, an ecdysone receptor protein obtained from various insects, and an ultraspiracle (USP) protein or the vertebrate homolog of USP, retinoid X
- 20 receptor protein (see Yao, et al. (1993) *Nature* 366, 476-479; Yao, et al., (1992) *Cell* 71, 63-72). However, the complex may also be a homodimer as detailed below. The functional ecdysteroid receptor complex may also include additional protein(s) such as immunophilins. Additional members of the steroid receptor family of proteins, known as transcriptional factors (such as DHR38 or *betaFTZ-1*), may also be ligand dependent or independent partners for EcR, USP, and/or RXR. Additionally, other
- 25 cofactors may be required such as proteins generally known as coactivators (also termed adapters or mediators). These proteins do not bind sequence-specifically to DNA and are not involved in basal transcription. They may exert their effect on transcription activation through various mechanisms, including stimulation of DNA-binding of activators, by affecting chromatin structure, or by mediating activator-initiation complex interactions. Examples of such coactivators include RIP140, TIF1,
- 30 RAP46/Bag-1, ARA70, SRC-1/NCoA-1, TIF2/GRIP/NCoA-2, ACTR/AIB1/RAC3/pCIP as well as the promiscuous coactivator C response element B binding protein, CBP/p300 (for review see Glass et al., *Curr. Opin. Cell Biol.* 9: 222-232, 1997). Also, protein cofactors generally known as corepressors (also known as repressors, silencers, or silencing mediators) may be required to effectively inhibit
- transcriptional activation in the absence of ligand. These corepressors may interact with the unliganded
- 35 ecdysone receptor to silence the activity at the response element. Current evidence suggests that the binding of ligand changes the conformation of the receptor, which results in release of the corepressor and recruitment of the above described coactivators, thereby abolishing their silencing activity.

Examples of corepressors include N-CoR and SMRT (for review, see Horwitz et al. Mol Endocrinol. 10: 1167-1177, 1996). These cofactors may either be endogenous within the cell or organism, or may be added exogenously as transgenes to be expressed in either a regulated or unregulated fashion.

Homodimer complexes of the ecdysone receptor protein, USP, or RXR may also be functional under
5 some circumstances.

The ecdysone receptor complex typically includes proteins that are members of the nuclear receptor superfamily wherein all members are generally characterized by the presence of an amino-terminal transactivation domain, a DNA binding domain ("DBD"), and a ligand binding domain ("LBD") separated from the DBD by a hinge region. As used herein, the term "DNA binding domain"
10 comprises a minimal polypeptide sequence of a DNA binding protein, up to the entire length of a DNA binding protein, so long as the DNA binding domain functions to associate with a particular response element. Members of the nuclear receptor superfamily are also characterized by the presence of four or five domains: A/B, C, D, E, and in some members F (see US patent 4,981,784 and Evans, *Science* 240: 889-895 (1988)). The "A/B" domain corresponds to the transactivation domain, "C" corresponds to the
15 DNA binding domain, "D" corresponds to the hinge region, and "E" corresponds to the ligand binding domain. Some members of the family may also have another transactivation domain on the carboxy-terminal side of the LBD corresponding to "F".

The DBD is characterized by the presence of two cysteine zinc fingers between which are two amino acid motifs, the P-box and the D-box, which confer specificity for ecdysone response elements.
20 These domains may be either native, modified, or chimeras of different domains of heterologous receptor proteins. This EcR receptor, like a subset of the steroid receptor family, also possesses less well-defined regions responsible for heterodimerization properties. Because the domains of EcR, USP, and RXR are modular in nature, the LBD, DBD, and transactivation domains may be interchanged.

Gene switch systems are known that incorporate components from the ecdysone receptor
25 complex. However, in these known systems, whenever EcR is used it is associated with native or modified DNA binding domains and transactivation domains on the same molecule. USP or RXR are typically used as silent partners. Applicants have previously shown that when DNA binding domains and transactivation domains are on the same molecule the background activity in the absence of ligand is high and that such activity is dramatically reduced when DNA binding domains and transactivation
30 domains are on different molecules, that is, on each of two partners of a heterodimeric or homodimeric complex (see PCT/US01/09050). This two-hybrid system also provides improved sensitivity to non-steroidal ligands for example, diacylhydrazines, when compared to steroidal ligands for example, ponasterone A ("PonA") or muristerone A ("MurA"). That is, when compared to steroids, the non-steroidal ligands provide higher activity at a lower concentration. In addition, since transactivation
35 based on EcR gene switches is often cell-line dependent, it is easier to tailor switching systems to obtain maximum transactivation capability for each application. Furthermore, the two-hybrid system avoids some side effects due to overexpression of RXR that often occur when unmodified RXR is used as a

switching partner. In a specific embodiment of the two-hybrid system, native DNA binding and transactivation domains of EcR or RXR are eliminated and as a result, these hybrid molecules have less chance of interacting with other steroid hormone receptors present in the cell resulting in reduced side effects.

5 Applicants have previously shown that an ecdysone receptor in partnership with a dipteran (fruit fly *Drosophila melanogaster*) or a lepidopteran (spruce bud worm *Choristoneura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor in partnership with a vertebrate retinoid X receptor (RXR) is inducible in mammalian cells (pending application PCT/US01/09050). Applicants have now made the surprising discovery that the ultraspiracle
10 protein of *Locusta migratoria* ("LmUSP") and the RXR homolog 1 and RXR homolog 2 of the ixodid tick *Amblyomma americanum* ("AmaRXR1" and "AmaRXR2", respectively) can function similar to vertebrate retinoid X receptor (RXR) in an inducible ecdysone receptor-based inducible gene expression system. Thus, Applicants' findings that LmUSP, AmaRXR1, AmaRXR2, and their non-Dipteran, non-Lepidopteran homologs including, but not limited to: fiddler crab *Celuca pugilator* RXR homolog
15 ("CpRXR"), beetle *Tenebrio molitor* RXR homolog ("TmRXR"), honeybee *Apis mellifera* RXR homolog ("AmRXR"), and aphid *Myzus persicae* RXR homolog ("MpRXR"), all of which are referred to herein collectively as invertebrate RXRs, can be substituted for vertebrate RXR in ecdysone receptor-based inducible gene expression systems can only be regarded as unexpected and surprising. As described herein, Applicants' novel ecdysone receptor/invertebrate RXR -based inducible gene
20 expression system provides an improved inducible gene expression system in yeast and mammalian cells that is characterized by increased ligand sensitivity and magnitude of transactivation.

In particular, Applicants describe herein a novel two-hybrid system that comprises an invertebrate RXR ligand binding domain. This novel gene expression system demonstrates for the first time that an invertebrate ultraspiracle protein/RXR homolog can function as a component of an
25 inducible EcR-based inducible gene expression system in yeast and mammalian cells. As discussed herein, this finding is both unexpected and surprising.

Specifically, Applicants' invention relates to a gene expression modulation system comprising:
a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a
30 DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide comprising i) a transactivation domain; and ii) an invertebrate retinoid X receptor ligand binding domain.

35 The present invention also relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a

DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an invertebrate retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide

5 comprising i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

The present invention also relates to a gene expression modulation system according to the present invention further comprising c) a third gene expression cassette comprising: i) a response element to which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose

1.0 expression is to be modulated.

In a specific embodiment, the gene whose expression is to be modulated is a homologous gene with respect to the host cell. In another specific embodiment, the gene whose expression is to be modulated is a heterologous gene with respect to the host cell.

The ligands for use in the present invention as described below, when combined with the ligand

1.5 binding domains of an EcR and an invertebrate RXR, which in turn are bound to the response element linked to a gene, provide the means for external temporal regulation of expression of the gene. The binding mechanism or the order in which the various components of this invention bind to each other, that is, for example, ligand to receptor, first hybrid polypeptide to response element, second hybrid polypeptide to promoter, etc., is not critical. Binding of the ligand to the ligand binding domains of an

2.0 EcR and invertebrate RXR enables expression or suppression of the gene. This mechanism does not exclude the potential for ligand binding to EcR or invertebrate RXR, and the resulting formation of active homodimer complexes (e.g. EcR + EcR or invertebrate RXR + invertebrate RXR). Preferably, one or more of the receptor domains is varied producing a chimeric or hybrid gene switch. Typically, one or more of the three domains, DBD, LBD, and transactivation domain, may be chosen from a source

2.5 different than the source of the other domains so that the hybrid genes and the resulting hybrid proteins are optimized in the chosen host cell or organism for transactivating activity, complementary binding of the ligand, and recognition of a specific response element. In addition, the response element itself can be modified or substituted with response elements for other DNA binding protein domains such as the GAL-4 protein from yeast (see Sadowski, et al., (1988) *Nature* 335: 563-564) or LexA protein from

3.0 *Escherichia coli* (see Brent and Ptashne, (1985), *Cell* 43: 729-736), or synthetic response elements specific for targeted interactions with proteins designed, modified, and selected for such specific interactions (see, for example, Kim, et al. (1997), *Proc. Natl. Acad. Sci., USA* 94: 3616-3620) to accommodate hybrid receptors. Another advantage of two-hybrid systems is that they allow choice of a promoter used to drive the gene expression according to a desired end result. Such double control can be

3.5 particularly important in areas of gene therapy, especially when cytotoxic proteins are produced, because both the timing of expression as well as the cells wherein expression occurs can be controlled. When genes, operably linked to a suitable promoter, are introduced into the cells of the subject, expression of

the exogenous genes is controlled by the presence of the system of this invention. Promoters may be constitutively or inducibly regulated or may be tissue-specific (that is, expressed only in a particular type of cells) or specific to certain developmental stages of the organism.

5 GENE EXPRESSION CASSETTES OF THE INVENTION

The novel EcR/invertebrate RXR-based inducible gene expression system of the invention comprises gene expression cassettes that are capable of being expressed in a host cell, wherein the gene expression cassettes each comprise a polynucleotide encoding a hybrid polypeptide. Thus, Applicants' invention also provides novel gene expression cassettes for use in the gene expression system of the
10 invention.

Specifically, the present invention provides a gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide. In particular, the present invention provides a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding
15 domain that recognizes a response element, or ii) a transactivation domain; and an ecdysone receptor ligand binding domain or an invertebrate retinoid X receptor ligand binding domain.

In a specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain that recognizes a response element and an EcR ligand binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide
20 comprising a DNA-binding domain that recognizes a response element and an invertebrate RXR ligand binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain and an EcR ligand binding domain.

In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide
25 comprising a transactivation domain and an invertebrate RXR ligand binding domain.

In a preferred embodiment, the ligand binding domain (LBD) is an EcR LBD, an invertebrate RXR LBD, or a related steroid/thyroid hormone nuclear receptor family member LBD, or analogs, combinations, or modifications thereof. In a specific embodiment, the LBD is from an EcR or an invertebrate RXR. In another specific embodiment, the LBD is from a truncated EcR LBD or a
30 truncated invertebrate RXR LBD. A truncation mutation may be made by any method used in the art, including but not limited to restriction endonuclease digestion/deletion, PCR-mediated/oligonucleotide-directed deletion, chemical mutagenesis, DNA strand breakage, and the like.

The EcR may be an invertebrate EcR, preferably selected from the class Arthropod. Preferably the EcR is selected from the group consisting of a Lepidopteran EcR, a Dipteran EcR, an Orthopteran
35 EcR, a Homopteran EcR and a Hemipteran EcR. More preferably, the EcR for use is a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR"), a beetle *Tenebrio molitor* EcR ("TmEcR"), a *Manduca sexta* EcR ("MsEcR"), a *Heliothis virescens* EcR ("HvEcR"), a midge *Chironomus tentans* EcR ("CtEcR"), a

silk moth *Bombyx mori* EcR ("BmEcR"), a fruit fly *Drosophila melanogaster* EcR ("DmEcR"), a mosquito *Aedes aegypti* EcR ("AaEcR"), a blowfly *Lucilia capitata* ("LcEcR"), a blowfly *Lucilia cuprina* EcR ("LucEcR"), a Mediterranean fruit fly *Ceratitis capitata* EcR ("CcEcR"), a locust *Locusta migratoria* EcR ("LmEcR"), an aphid *Myzus persicae* EcR ("MpEcR"), a fiddler crab *Celuca pugilator* EcR ("CpEcR"), an ixodid tick *Amblyomma americanum* EcR ("AmaEcR"), a whitefly *Bamecia argentifoli* EcR ("BaEcR", SEQ ID NO: 57), or a leafhopper *Nephotetix cincticeps* EcR ("NcEcR", SEQ ID NO: 58). Most preferably, the LBD is from spruce budworm (*Choristoneura fumiferana*) EcR ("CfEcR"), fruit fly *Drosophila melanogaster* EcR ("DmEcR"), whitefly *Bamecia argentifoli* EcR ("BaEcR"), leafhopper *Nephotetix cincticeps* EcR ("NcEcR"), beetle *Tenebrio molitor* EcR ("TmEcR"), or ixodid tick *Amblyomma americanum* EcR ("AmaEcR").

In a specific embodiment, the LBD is from a truncated EcR polypeptide. The EcR polypeptide truncation results in a deletion of at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, or 265 amino acids. Preferably, the EcR polypeptide truncation results in a deletion of at least a partial polypeptide domain. More preferably, the EcR polypeptide truncation results in a deletion of at least an entire polypeptide domain. In a specific embodiment, the EcR polypeptide truncation results in a deletion of at least an A/B-domain, a C-domain, a D-domain, an F-domain, an A/B/C-domains, an A/B/1/2-C-domains, an A/B/C/D-domains, an A/B/C/D/F-domains, an A/B/F-domains, an A/B/C/F-domains, a partial E domain, or a partial F domain. A combination of several complete and/or partial domain deletions may also be performed.

In one embodiment, the ecdysone receptor ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 (DmEcR-EF), SEQ ID NO: 3 (CfEcR-DE), and SEQ ID NO: 4 (DmEcR-DE). In a preferred embodiment, the ecdysone receptor ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 (CfEcR-EF), SEQ ID NO: 53 (CfEcR-DEF), and SEQ ID NO: 45 (CfEcR-CDEF).

In one embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (DmEcR-EF), SEQ ID NO: 7 (CfEcR-DE), and SEQ ID NO: 8 (DmEcR-DE). In a preferred embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 43 (CfEcR-DEF), and SEQ ID NO: 59 (CfEcR-CDEF).

Preferably, the invertebrate RXR polypeptide is a locust *Locusta migratoria* ultraspiracle polypeptide ("LmUSP"), an ixodid tick *Amblyomma americanum* RXR homolog 1 ("AmaRXR1"), a ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), a fiddler crab *Celuca pugilator* RXR homolog ("CpRXR"), a beetle *Tenebrio molitor* RXR homolog ("TmRXR"), a honeybee *Apis mellifera* RXR homolog ("AmRXR"), an aphid *Myzus persicae* RXR homolog ("MpRXR"), or a non-

Dipteran/non-Lepidopteran RXR homolog.

In a specific embodiment, the LBD is from a truncated invertebrate RXR. The invertebrate RXR polypeptide truncation results in a deletion of at least 1, 2, 3, 4, 5, 6, 8, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, or 240 amino acids. Preferably, the invertebrate RXR polypeptide truncation results in a deletion of at least a partial polypeptide domain. More preferably, the invertebrate RXR polypeptide truncation results in a deletion of at least an entire polypeptide domain. In a specific embodiment, the invertebrate RXR polypeptide truncation results in a deletion of at least a partial E-domain, a complete E-domain, a partial F-domain, a complete F-domain, an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an EF-domain β -pleated sheet, an A/B-domain, a C-domain, a D-domain, A/B/C-domains, A/B/1/2-C-domains, A/B/C/D-domains, A/B/C/D/F-domains, A/B/F-domains, or A/B/C/F-domains. A combination of several complete and/or partial domain deletions may also be performed.

In a preferred embodiment, the invertebrate RXR ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), SEQ ID NO: 14 (AmRXR-EF), SEQ ID NO: 15 (LmUSP-EF, BamHI-deleted), SEQ ID NO: 16 (AmaRXR1-EF, BamHI-deleted), SEQ ID NO: 17 (AmaRXR2-EF, BamHI-deleted), SEQ ID NO: 18 (CpRXR-EF, BamHI-deleted), SEQ ID NO: 19 (TmRXR-EF, BamHI-deleted), and SEQ ID NO: 20 (AmRXR-EF, BamHI-deleted).

In another preferred embodiment, the invertebrate RXR ligand binding domain comprises a polypeptide sequence selected from the group consisting of SEQ ID NO: 21 (LmUSP-EF), SEQ ID NO: 22 (AmaRXR1-EF), SEQ ID NO: 23 (AmaRXR2-EF), SEQ ID NO: 24 (CpRXR-EF), SEQ ID NO: 25 (TmRXR-EF), SEQ ID NO: 26 (AmRXR-EF), SEQ ID NO: 27 (LmUSP-EF, BamHI-deleted), SEQ ID NO: 28 (AmaRXR1-EF, BamHI-deleted), SEQ ID NO: 29 (AmaRXR2-EF, BamHI-deleted), SEQ ID NO: 30 (CpRXR-EF, BamHI-deleted), SEQ ID NO: 31 (TmRXR-EF, BamHI-deleted), and SEQ ID NO: 32 (AmRXR-EF, BamHI-deleted).

For purposes of this invention, EcR and invertebrate RXR also include synthetic and chimeric EcR and invertebrate RXR and their homologs.

The DNA binding domain can be any DNA binding domain with a known response element, including synthetic and chimeric DNA binding domains, or analogs, combinations, or modifications thereof. Preferably, the DBD is a GAL4 DBD, a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, or a yeast put DBD. More preferably, the DBD is a GAL4 DBD [SEQ ID NO: 33 (polynucleotide) or SEQ ID NO: 34 (polypeptide)] or a LexA DBD [(SEQ ID NO: 35 (polynucleotide) or SEQ ID NO: 36 (polypeptide))].

The transactivation domain (abbreviated "AD" or "TA") may be any steroid/thyroid hormone nuclear receptor AD, synthetic or chimeric AD, polyglutamine AD, basic or acidic amino acid AD, a VP16 AD, a GAL4 AD, an NF- κ B AD, a BP64 AD, a B42 acidic activation domain (B42AD), or an analog, combination, or modification thereof. In a specific embodiment, the AD is a synthetic or
 5 chimeric AD, or is obtained from a VP16, GAL4, NF- κ B, or B42 acidic activation domain AD. Preferably, the AD is a VP16 AD [SEQ ID NO: 37 (polynucleotide) or SEQ ID NO: 38 (polypeptide)] or a B42 AD [SEQ ID NO: 39 (polynucleotide) or SEQ ID NO: 40 (polypeptide)].

In a preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence
 10 selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) and a LexA DBD (SEQ ID NO: 35), and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53, and SEQ ID NO: 45.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group
 15 consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence
 20 selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) or a LexA DBD (SEQ ID NO: 35) and an invertebrate RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group
 25 consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate RXR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26,
 30 SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of
 35 SEQ ID NO: 37 or SEQ ID NO: 39, and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53, and SEQ ID NO: 45.

In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide

comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 40, and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

5 In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 37 or SEQ ID NO: 39, and an invertebrate RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

10 In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 40 and an invertebrate RXR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID
15 NO: 31, and SEQ ID NO: 32.

The response element ("RE") may be any response element with a known DNA binding domain, or an analog, combination, or modification thereof. A single RE may be employed or multiple REs, either multiple copies of the same RE or two or more different REs, may be used in the present invention. In a specific embodiment, the RE is an RE from GAL4 ("GAL4RE"), LexA, a steroid/thyroid
20 hormone nuclear receptor RE, or a synthetic RE that recognizes a synthetic DNA binding domain. Preferably, the RE is a GAL4RE comprising a polynucleotide sequence of SEQ ID NO: 41 or a LexA RE (operon, "op") comprising a polynucleotide sequence of SEQ ID NO: 42 ("2XLexAopRE"). Preferably, the first hybrid protein is substantially free of a transactivation domain and the second hybrid protein is substantially free of a DNA binding domain. For purposes of this invention, "substantially
25 free" means that the protein in question does not contain a sufficient sequence of the domain in question to provide activation or binding activity.

Thus, the present invention also relates to a gene expression cassette comprising: i) a response element comprising a domain to which a polypeptide comprising a DNA binding domain binds; ii) a promoter that is activated by a polypeptide comprising a transactivation domain; and iii) a gene whose
30 expression is to be modulated.

Genes of interest for use in Applicants' gene expression cassettes may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic
35 acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

Examples of genes of interest for use in Applicants' gene expression cassettes include, but are not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferons, insulin, erythropoietin, clotting factors, other blood factors or
5 components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, functional genomics, and proteomics analyses and applications, and the like.

POLYNUCLEOTIDES OF THE INVENTION

The novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression
1 0 system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of an EcR-based gene expression system to modulate the expression of a gene within a host cell.

1 5 Thus, the present invention provides an isolated polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain according to the invention.

The present invention also relates to an isolated polynucleotide that encodes a truncated EcR or a
2 0 truncated invertebrate RXR polypeptide comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated polynucleotide encoding an EcR or an invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity that is useful in modulating gene expression in a host cell.

In a specific embodiment, the isolated truncated EcR polynucleotide comprises a polynucleotide
2 5 sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

In another specific embodiment, the isolated truncated EcR polynucleotide encodes a truncated ecdysone receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

In another specific embodiment, the isolated truncated invertebrate RXR polynucleotide
3 0 according to the invention comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

In another specific embodiment, the isolated truncated invertebrate RXR polynucleotide according to the invention encodes a truncated invertebrate RXR polypeptide comprising an amino acid
3 5 sequence consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

In particular, the present invention relates to an isolated polynucleotide encoding an invertebrate

RXR polypeptide comprising a truncation mutation, wherein the mutation reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

In another specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

The present invention also relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation, wherein the mutation enhances ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

In another specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

The present invention also relates to an isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the mutated invertebrate retinoid X receptor polypeptide and a dimerization partner. Preferably, the isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), and SEQ ID NO: 14 (AmRXR-EF). In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. More preferably, the dimerization partner is an EcR polypeptide in which domain A/B has been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 43 (CfEcR-DEF) or SEQ ID NO: 59 (CfEcR-CDEF).

POLYPEPTIDES OF THE INVENTION

The novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of

an EcR-based gene expression system to modulate the expression of a gene within a host cell.

Thus, the present invention also relates to a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain according to the invention.

5 The present invention also relates to an isolated truncated EcR or an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated truncated EcR or an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity.

10 In a specific embodiment, the isolated truncated EcR polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

In another specific embodiment, the isolated truncated EcR polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

15 In another specific embodiment, the isolated truncated invertebrate RXR polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

In another specific embodiment, the isolated truncated invertebrate RXR polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

20 The present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide, wherein the polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

25 Thus, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

30 In a specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

35 In another specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

In addition, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the invertebrate RXR

polypeptide.

The present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

In another specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

The present invention also relates to an isolated invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the mutated invertebrate retinoid X receptor polypeptide and a dimerization partner. Preferably, the isolated invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), and SEQ ID NO: 14 (AmRXR-EF). More preferably, the isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21 (LmUSP-EF), SEQ ID NO: 22 (AmaRXR1-EF), SEQ ID NO: 23 (AmaRXR2-EF), SEQ ID NO: 24 (CpRXR-EF), SEQ ID NO: 25 (TmRXR-EF), and SEQ ID NO: 26 (AmRXR-EF).

In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. More preferably, the dimerization partner is an EcR polypeptide in which domain A/B has been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 43 (CfEcR-DEF) or SEQ ID NO: 59 (CfEcR-CDEF).

METHOD OF MODULATING GENE EXPRESSION OF THE INVENTION

Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; wherein the gene to be modulated is a component of a gene expression cassette comprising: i) a response element comprising a domain recognized by the DNA binding domain of the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated, whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

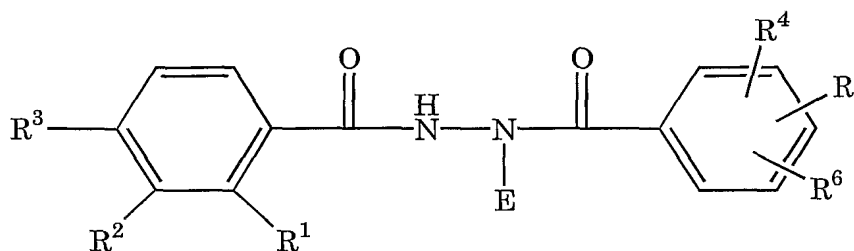
The invention also provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain recognized by the DNA binding domain from the first hybrid polypeptide; 5 ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

Genes of interest for expression in a host cell using Applicants' methods may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or 10 protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

15 Examples of genes of interest for expression in a host cell using Applicants' methods include, but are not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferons, insulin, erythropoietin, clotting factors, other blood factors or components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, 20 functional genomics, and proteomics analyses and applications, and the like.

Acceptable ligands are any that modulate expression of the gene when binding of the DNA binding domain of the two-hybrid system to the response element in the presence of the ligand results in activation or suppression of expression of the genes. Preferred ligands include ponasterone, muristerone A, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed 25 in U. S. Patents No. 6,013,836; 5,117,057; 5,530,028; and 5,378,726; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 461,809; N-alkyl-N,N'-diaroylhydrazines such as those disclosed in U. S. Patent No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed in European Application No. 234,994; N-aroyl-N-alkyl-N'-aroylhydrazines such as those described in U. S. Patent No. 4,985,461; each of which is incorporated herein by reference and other similar materials 30 including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, and the like.

In a preferred embodiment, the ligand for use in Applicants' method of modulating expression of gene is a compound of the formula:



wherein:

E is a (C₄-C₆)alkyl containing a tertiary carbon or a cyano(C₃-C₅)alkyl containing a tertiary carbon;

R¹ is H, Me, Et, i-Pr, F, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, SCN, or SCHF₂;

R² is H, Me, Et, n-Pr, i-Pr, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe₂, NEt₂, SMe, SEt, SOCF₃, OCF₂CF₂H, COEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, OCF₃, OCHF₂, O-i-Pr, SCN, SCHF₂, SOME, NH-CN, or joined with R³ and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R³ is H, Et, or joined with R² and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R⁴, R⁵, and R⁶ are independently H, Me, Et, F, Cl, Br, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

In another preferred embodiment, a second ligand may be used in addition to the first ligand discussed above in Applicants' method of modulating expression of a gene, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of retinoic acid.

Applicants' invention provides for modulation of gene expression in prokaryotic and eukaryotic host cells. Thus, the present invention also relates to a method for modulating gene expression in a host cell selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell. Preferably, the host cell is a yeast cell, a hamster cell, a mouse cell, a monkey cell, or a human cell.

Expression in transgenic host cells may be useful for the expression of various polypeptides of interest including but not limited to therapeutic polypeptides, pathway intermediates; for the modulation of pathways already existing in the host for the synthesis of new products heretofore not possible using the host; cell based assays; functional genomics assays, biotherapeutic protein production, proteomics assays, and the like. Additionally the gene products may be useful for conferring higher growth yields of the host or for enabling an alternative growth mode to be utilized.

HOST CELLS AND NON-HUMAN ORGANISMS OF THE INVENTION

As described above, the gene expression modulation system of the present invention may be used to modulate gene expression in a host cell. Expression in transgenic host cells may be useful for the expression of various genes of interest. Thus, Applicants' invention provides an isolated host cell

5 comprising a gene expression system according to the invention. The present invention also provides an isolated host cell comprising a gene expression cassette according to the invention. Applicants' invention also provides an isolated host cell comprising a polynucleotide or a polypeptide according to the invention. The isolated host cell may be either a prokaryotic or a eukaryotic host cell.

Preferably, the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a
10 yeast cell, an animal cell, and a mammalian cell. Examples of preferred host cells include, but are not limited to, fungal or yeast species such as *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, or bacterial species such as those in the genera *Synechocystis*, *Synechococcus*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*,
15 animal, and mammalian host cells.

In a specific embodiment, the host cell is a yeast cell selected from the group consisting of a *Saccharomyces*, a *Pichia*, and a *Candida* host cell.

In another specific embodiment, the host cell is a hamster cell.

In another specific embodiment, the host cell is a murine cell.

20 In another specific embodiment, the host cell is a monkey cell.

In another specific embodiment, the host cell is a human cell.

Host cell transformation is well known in the art and may be achieved by a variety of methods including but not limited to electroporation, viral infection, plasmid/vector transfection, non-viral vector mediated transfection, particle bombardment, and the like. Expression of desired gene products involves
25 culturing the transformed host cells under suitable conditions and inducing expression of the transformed gene. Culture conditions and gene expression protocols in prokaryotic and eukaryotic cells are well known in the art (see General Methods section of Examples). Cells may be harvested and the gene products isolated according to protocols specific for the gene product.

In addition, a host cell may be chosen which modulates the expression of the inserted
30 polynucleotide, or modifies and processes the polypeptide product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification [*e.g.*, glycosylation, cleavage (*e.g.*, of signal sequence)] of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to
35 produce a non-glycosylated core protein product. However, a polypeptide expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of a heterologous protein.

Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting, the polypeptide's activity. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.

Applicants' invention also relates to a non-human organism comprising an isolated host cell
5 according to the invention. Preferably, the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal. More preferably, the non-human organism is a yeast, a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, or a chimpanzee.

In a specific embodiment, the non-human organism is a yeast selected from the group consisting
10 of *Saccharomyces*, *Pichia*, and *Candida*.

In another specific embodiment, the non-human organism is a *Mus musculus* mouse.

MEASURING GENE EXPRESSION/TRANSCRIPTION

One useful measurement of Applicants' methods of the invention is that of the transcriptional
15 state of the cell including the identities and abundances of RNA, preferably mRNA species. Such measurements are conveniently conducted by measuring cDNA abundances by any of several existing gene expression technologies.

Nucleic acid array technology is a useful technique for determining differential mRNA
expression. Such technology includes, for example, oligonucleotide chips and DNA microarrays. These
20 techniques rely on DNA fragments or oligonucleotides which correspond to different genes or cDNAs which are immobilized on a solid support and hybridized to probes prepared from total mRNA pools extracted from cells, tissues, or whole organisms and converted to cDNA. Oligonucleotide chips are arrays of oligonucleotides synthesized on a substrate using photolithographic techniques. Chips have been produced which can analyze for up to 1700 genes. DNA microarrays are arrays of DNA samples,
25 typically PCR products, that are robotically printed onto a microscope slide. Each gene is analyzed by a full-or partial-length target DNA sequence. Microarrays with up to 10,000 genes are now routinely prepared commercially. The primary difference between these two techniques is that oligonucleotide chips typically utilize 25-mer oligonucleotides which allow fractionation of short DNA molecules whereas the larger DNA targets of microarrays, approximately 1000 base pairs, may provide more
30 sensitivity in fractionating complex DNA mixtures.

Another useful measurement of Applicants' methods of the invention is that of determining the translation state of the cell by measuring the abundances of the constituent protein species present in the cell using processes well known in the art.

Where identification of genes associated with various physiological functions is desired, an
35 assay may be employed in which changes in such functions as cell growth, apoptosis, senescence, differentiation, adhesion, binding to a specific molecules, binding to another cell, cellular organization, organogenesis, intracellular transport, transport facilitation, energy conversion, metabolism, myogenesis,

neurogenesis, and/or hematopoiesis is measured.

In addition, selectable marker or reporter gene expression may be used to measure gene expression modulation using Applicants' invention.

Other methods to detect the products of gene expression are well known in the art and include
5 Southern blots (DNA detection), dot or slot blots (DNA, RNA), northern blots (RNA), RT-PCR (RNA), western blots (polypeptide detection), and ELISA (polypeptide) analyses. Although less preferred, labeled proteins can be used to detect a particular nucleic acid sequence to which it hybridizes.

In some cases it is necessary to amplify the amount of a nucleic acid sequence. This may be carried out using one or more of a number of suitable methods including, for example, polymerase chain
10 reaction ("PCR"), ligase chain reaction ("LCR"), strand displacement amplification ("SDA"), transcription-based amplification, and the like. PCR is carried out in accordance with known techniques in which, for example, a nucleic acid sample is treated in the presence of a heat stable DNA polymerase, under hybridizing conditions, with one pair of oligonucleotide primers, with one primer hybridizing to one strand (template) of the specific sequence to be detected. The primers are sufficiently
15 complementary to each template strand of the specific sequence to hybridize therewith. An extension product of each primer is synthesized and is complementary to the nucleic acid template strand to which it hybridized. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample may be analyzed as described above to assess whether the
20 sequence or sequences to be detected are present.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention.

EXAMPLES

25

GENERAL METHODS

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. (1989) (Maniatis) and by T. J.
30 Silhavy, M. L. Bennis, and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience (1987).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in
35 *Manual of Methods for General Bacteriology* (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in *Biotechnology: A Textbook of*

Industrial Microbiology, Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of host cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

5 Manipulations of genetic sequences may be accomplished using the suite of programs available from the Genetics Computer Group Inc. (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI). Where the GCG program "Pileup" is used the gap creation default value of 12, and the gap extension default value of 4 may be used. Where the CGC "Gap" or "Bestfit" program is used the default gap creation penalty of 50 and the default gap extension penalty of 3 may be used. In
10 any case where GCG program parameters are not prompted for, in these or any other GCG program, default values may be used.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "μl" means microliter(s), "ml" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "μg" means microgram(s), "mg" means
15 milligram(s), "A" means adenine or adenosine, "T" means thymine or thymidine, "G" means guanine or guanosine, "C" means cytidine or cytosine, "x g" means times gravity, "nt" means nucleotide(s), "aa" means amino acid(s), "bp" means base pair(s), "kb" means kilobase(s), "k" means kilo, "μ" means micro, and "°C" means degrees Celsius.

20 EXAMPLE 1

Applicants' EcR/invertebrate RXR-based inducible gene modulation system is useful in various applications including gene therapy, expression of proteins of interest in host cells, production of transgenic organisms, and cell-based assays. In various cellular backgrounds, including mammalian
25 cells, invertebrate EcR heterodimerizes with vertebrate RXR and, upon binding of ligand, transactivates genes under the control of ecdysone response elements. Applicants have made the surprising discovery that invertebrate RXR can substitute for vertebrate RXR and provide a novel inducible gene expression system for yeast and animal cell applications. This Example describes the construction of several gene expression cassettes for use in the EcR-based inducible gene expression system of the invention.

30 Applicants constructed several EcR-based gene expression cassettes based on the spruce budworm *Choristoneura fumiferana* EcR ("CfEcR"), *C. fumiferana* ultraspiracle ("CfUSP"), *Drosophila melanogaster* USP ("DmUSP"), mouse *Mus musculus* retinoid X receptor α ("MmRXRα"), locust *Locusta migratoria* USP ("LmUSP"), an invertebrate homolog of vertebrate RXR, *Amblyomma americanum* RXR homolog 1 ("AmaRXR1"), an invertebrate homolog of vertebrate RXR, and
35 *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), an invertebrate homolog of vertebrate RXR. The prepared receptor constructs comprise a ligand binding domain of either an EcR, a vertebrate RXR, an invertebrate USP, or an invertebrate RXR; and a GAL4 or LexA DNA binding domain (DBD) or a

VP16 or B42 acidic activator transactivation domain (AD). The reporter constructs include a reporter gene, luciferase or LacZ, operably linked to a synthetic promoter construct that comprises either a GAL4 response element or a LexA response element to which the Gal4 DBD or LexA DBD binds, respectively.

Various combinations of these receptor and reporter constructs were cotransfected into mammalian cells as described in Examples 2-9 *infra*.

Gene Expression Cassettes: Ecdysone receptor-based gene expression cassettes (switches) were constructed as followed, using standard cloning methods available in the art. The following is brief description of preparation and composition of each switch used in the Examples described herein.

1.1 - GAL4CfEcR-CDEF/VP16MmRXR α -DEF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 45) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The DEF domains from mouse (*Mus musculus*) RXR α ("MmRXR α -DEF"; SEQ ID NO: 47) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.2 - GAL4CfEcR-CDEF/VP16MmRXR α -EF: This construct was prepared in the same way as in switch 1.1 above except MmRXR α -DEF was replaced with MmRXR α -EF (SEQ ID NO: 50).

1.3 - GAL4CfEcR-CDEF/VP16CfUSP-DEF: This construct was prepared in the same way as in switch 1.1 above except MmRXR α -DEF was replaced with the D, E and F domains from spruce budworm USP ("CfUSP-DEF"; SEQ ID NO: 51). The constructs used in this example are similar to those disclosed in U. S. Patent No. 5,880,333 except that *Choristoneura fumiferana* USP rather than *Drosophila melanogaster* USP was utilized.

1.4 - GAL4CfEcR-CDEF/VP16LmUSP-DEF: This construct was prepared in the same way as in switch 1.1 above except MmRXR α -DEF was replaced with the D, E and F domains of *Locusta migratoria* ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52).

1.5 - GAL4CfEcR-DEF/VP16MmRXR α -DEF: This construct was prepared in the same way as switch 1.1 except CfEcR-CDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.6 - GAL4CfEcR-DEF/VP16MmRXR α -EF: This construct was prepared in the same way as switch 1.5 except MmRXR α -DEF was replaced with MmRXR α -EF (SEQ ID NO: 50).

1.7 - GAL4CfEcR-DEF/VP16CfUSP-DEF: This construct was prepared in the same way as in switch 1.5 above except MmRXR α -DEF was replaced with the D, E and F domains from spruce budworm *C. fumiferana* USP ("CfUSP-DEF"; SEQ ID NO: 51).

1.8 - GAL4CfEcR-DEF/VP16LmUSP-DEF: This construct was prepared in the same way as in switch 1.5 above except MmRXR α -DEF was replaced with the D, E, and F domains of *Locusta migratoria*

ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52).

1.9 - Gal4CfEcR-A/BCDEF/VP16LmUSP-DEF: The full-length spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-A/BCDEF"; SEQ ID NO: 54) was fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The DEF domains from *Locusta migratoria* ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.10 - Gal4CfEcR-1/2CDEF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-1/2CDEF (SEQ ID NO: 55).

1.11 - Gal4CfEcR-CDEF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-CDEF (SEQ ID NO: 45).

1.12 - Gal4CfEcR-DEF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.13 - Gal4CfEcR-EF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-EF (SEQ ID NO: 1).

1.14 - Gal4CfEcR-DE/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-CDEF was replaced with CfEcR-DE (SEQ ID NO: 3).

1.15 - Gal4CfEcR-A/BCDEF/VP16LmUSP-EF: The full-length spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-A/BCDEF"; SEQ ID NO: 54) was fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The EF domains from *Locusta migratoria* ultraspiracle ("LmUSP-EF"; SEQ ID NO: 9) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.16 - Gal4CfEcR-1/2CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-1/2CDEF (SEQ ID NO: 55).

1.17 - Gal4CfEcR-CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-CDEF (SEQ ID NO: 45).

1.18 - Gal4CfEcR-DEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.19 - Gal4CfEcR-EF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-EF (SEQ ID NO: 1).

- 1.20 - Gal4CfEcR-DEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-CDEF was replaced with CfEcR-DE (SEQ ID NO: 3).
- 1.21 - Gal4CfEcR-DEF/VP16AmaRXR1-EF: This construct was prepared in the same way as switch 1.18 except LmUSP-EF was replaced with the E and F domains of ixodid tick *Amblyomma americanum* RXR homolog 1 ("AmaRXR1-EF"; SEQ ID NO: 10).
- 1.22 - Gal4CfEcR-DEF/VP16AmaRXR2-EF: This construct was prepared in the same way as switch 1.21 except AmaRXR1-EF was replaced with the E and F domains of ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2-EF"; SEQ ID NO: 11).
- 1.23 - LexACfEcR-CDEF/VP16CfUSP-EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 45) were fused to a LexA DNA binding domain ("LexADNABD" or "LexADBD"; SEQ ID NO: 35) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The E and F domains from spruce budworm *C. fumiferana* USP ("CfUSP-EF"; SEQ ID NO: 56) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Eight consensus LexA response element binding sites ("8XLexAop"; comprising 4 copies of a LexA response element binding site comprising SEQ ID NO: 42) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).
- 1.24 - LexACfEcR-CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with LmUSP-EF (SEQ ID NO: 9).
- 1.25 - LexACfEcR-CDEF/VP16MmRXR α -EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with MmRXR α -EF (SEQ ID NO: 50).
- 1.26 - LexACfEcR-CDEF/VP16DmUSP-EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with the corresponding EF domains of DmUSP-EF (SEQ ID NO: 60).
- 1.27 - Gal4CfEcR-CDEF/B42LmUSP-EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 45) were fused to a GAL4 DNA binding domain ("GAL4DNABD" or "GAL4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The E and F domains from locust *Locusta migratoria* USP ("LmUSP-EF"; SEQ ID NO: 9) were fused to the transactivation domain from B42 ("B42AD"; SEQ ID NO: 39) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).
- 1.28 - LexACfEcR-CDEF/B42LmUSP-EF: This construct was prepared in the same way as switch 1.27 except the GAL4 DNA binding domain was replaced with a LexA DNA binding domain (SEQ ID NO: 35).
- 1.29 - GAL4CfEcR-DEF/VP16DmUSP-EF: This construct was prepared in the same way as switch 1.7 except CfUSP-DEF was replaced with the corresponding EF domains of DmUSP-EF (SEQ ID NO: 60).

1.30 - GAL4CfEcR-DEF/VP16CfUSP-EF: This construct was prepared in the same way as switch 1.7 except CfUSP-DEF was replaced with CfUSP-EF (SEQ ID NO: 56).

EXAMPLE 2

5

In a two-hybrid switch format, CfUSP and DmUSP in partnership with CfEcR are constitutively active in both yeast and mammalian cells. On the other hand, vertebrate RXR in partnership with CfEcR is a ligand dependent transactivator in mammalian cells. Applicants tested an invertebrate RXR, LmUSP in a two-hybrid format in mouse NIH3T3 cells to determine if it would function as a USP (constitutively) or as a vertebrate RXR (inducibly) in mammalian cells. Gal4:CfEcR-CDEF (Figure 1) or Gal4:CfEcR-DEF (Figure 2) were paired with VP16:MmRXR-DEF; VP16:MmRXR-EF; VP16:LmUSP-DEF; or VP16:CfUSP-EF and analyzed in mammalian cells. Briefly, gene induction potential (magnitude of induction) and ligand specificity and sensitivity were examined using two different ligands: a steroidal ligand (Ponasterone A, "PonA") and a non-steroidal ligand [N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine] in a dose-dependent induction of reporter gene expression in the transfected NIH3T3 cells. Reporter gene expression activities were assayed at 48 hours after ligand addition. Standard methods for culture and maintenance of the cells were followed.

Transfections: DNAs corresponding to the various switch constructs outlined in Example 1, specifically switches 1.1 through 1.8, were transfected into mouse NIH3T3 cells (ATCC) as follows. Cells were harvested when they reached 50% confluency and plated in 6-, 12- or 24- well plates at 125,000, 50,000, or 25,000 cells, respectively, in 2.5, 1.0, or 0.5 ml of growth medium containing 10% fetal bovine serum (FBS), respectively. The next day, the cells were rinsed with growth medium and transfected for four hours. Superfect™ (Qiagen Inc.) was found to be the best transfection reagent for 3T3 cells. For 12- well plates, 4 µl of Superfect™ was mixed with 100 µl of growth medium. 1.0 µg of reporter construct and 0.25 µg of each receptor construct of the receptor pair to be analyzed were added to the transfection mix. A second reporter construct was added [pTKRL (Promega), 0.1 µg/transfection mix] that comprises a *Renilla* luciferase gene operably linked and placed under the control of a thymidine kinase (TK) constitutive promoter and was used for normalization. The contents of the transfection mix were mixed in a vortex mixer and let stand at room temperature for 30 min. At the end of incubation, the transfection mix was added to the cells maintained in 400 µl growth medium. The cells were maintained at 37°C and 5% CO₂ for four hours. At the end of incubation, 500 µl of growth medium containing 20% FBS and either dimethylsulfoxide (DMSO; control) or a DMSO solution of 0.1, 1, 5, 10, and 50 µM PonA steroidal ligand or N-(2-ethyl-3-methoxybenzoyl)N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine non-steroidal ligand was added and the cells were maintained at 37°C and 5% CO₂ for 48 hours. The cells were harvested and reporter activity was assayed. The same procedure was followed for 6 and 24 well plates as well except all the reagents were doubled for 6 well plates and reduced to half for 24-well plates.

Ligands: The steroidal ligand Ponasterone A (PonA) was purchased from Sigma Chemical Company. The non-steroidal ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-t-butylhydrazine (GSTM-E non-steroidal ligand) is a synthetic stable ecdysteroid ligand synthesized at Rohm and Haas Company. All ligands were dissolved in DMSO and the final concentration of DMSO was maintained at 0.1% in both controls and treatments.

Reporter Assays: Cells were harvested 48 hours after adding ligands. 125, 250, or 500 μ l of passive lysis buffer (part of Dual-luciferaseTM reporter assay system from Promega Corporation) were added to each well of 24- or 12- or 6-well plates respectively. The plates were placed on a rotary shaker for 15 minutes. Twenty μ l of lysate were assayed. Luciferase activity was measured using Dual-luciferaseTM

- reporter assay system from Promega Corporation following the manufacturer's instructions. β -Galactosidase was measured using Galacto-StarTM assay kit from TROPIX following the manufacturer's instructions. All luciferase and β -galactosidase activities were normalized using *Renilla* luciferase as a standard. Fold activities were calculated by dividing normalized relative light units ("RLU") in ligand treated cells with normalized RLU in DMSO treated cells (untreated control).
- Results:** As shown in Figures 1 and 2, LmUSP in partnership with CfEcR functions as a ligand-inducible gene expression system in mammalian cells. This result is surprising since Applicants' previous experiments with CfUSP and DmUSP in partnership with CfEcR demonstrated constitutive expression activity (see PCT/US01/09050 application and Figures 1 and 2 for CfUSP results; DmUSP results are not shown). In addition, LmUSP worked better than vertebrate RXR as a CfEcR partner. In particular, both the sensitivity, *i.e.* the concentration of ligand required for transactivation, and the magnitude of transactivation were increased with LmUSP compared to vertebrate RXR. Thus, Applicants have demonstrated for the first time that invertebrate RXRs can function effectively in partnership with an ecdysone receptor in an inducible gene expression system in mammalian cells. This EcR/invertebrate RXR inducible gene expression system is an improvement over the EcR/vertebrate RXR gene expression system since less ligand is required for transactivation and increased levels of transactivation can be achieved.

- Based upon Applicant's discovery described herein, one of ordinary skill in the art is able to predict that other invertebrate RXRs and their homologs, with the exception of Dipteran RXR homologs (example DmUSP) and Lepidopteran RXR homologs (example CfUSP), will also function in Applicants' EcR/invertebrate RXR-based inducible gene expression system. In addition, one of ordinary skill in the art is also able to predict that Applicants' novel inducible gene expression system will also work to modulate gene expression in yeast cells. Since the Dipteran RXR homolog/ and Lepidopteran RXR homolog/EcR gene expression systems function constitutively in yeast cells (data not shown), similar to how they function in mammalian cells, and Applicants have shown herein that non-Dipteran and non-Lepidopteran invertebrate RXRs function inducibly in partnership with an EcR in mammalian cells, the EcR/invertebrate RXR-based gene expression system is also predicted to function inducibly in yeast cells. Thus, the EcR/invertebrate RXR inducible gene expression system of the present invention is

useful in applications where modulation of gene expression levels is desired in both yeast and mammalian cells. Further, there is no reason not to expect that the present invention would also work in other cells.

5

EXAMPLE 3

This Example describes the comparison of vertebrate RXR and invertebrate RXR-based two-hybrid gene expression systems comprising full length or truncated EcR, vertebrate RXR, and invertebrate RXR polypeptides. An amino acid sequence alignment, comparing the EF domains of
 10 twelve different vertebrate and invertebrate RXRs is shown in Figure 3A and B. As described below, Applicants compared different GAL4/CfEcR-based switches comprising MmRXR α -EF (a vertebrate RXR), LmUSP-EF (an invertebrate RXR), AmaRXR1-EF (an invertebrate RXR), and AmaRXR2-EF (an invertebrate RXR) fused to a VP16 activation domain to identify the receptors that give a switch with a)
 15 highly sensitive to ligand concentration; and/or d) minimum cross-talk among ligands and receptors in mammalian cells.

Briefly, full-length EcR and truncated EcRs, created by a truncation mutation at the junctions of A/B, C, D, E and F domains and fused to a GAL4 DNA binding domain encoding polynucleotide (SEQ ID NO: 33) as described in Example 1 above. A VP16 activation domain encoding polynucleotide (SEQ
 20 ID NO: 37) was fused to the E and F domains of MmRXR α , LmUSP, AmaRXR1, and AmaRXR2 as described in Example 1. The resulting hybrid EcR/vertebrate or invertebrate RXR-encoding gene expression cassettes were assayed in NIH3T3 cells in pairwise comparisons. Plasmid pFRLUC (Stratagene) encoding a luciferase polypeptide was used as a reporter gene construct and pTKRL (Promega) encoding a *Renilla* luciferase polypeptide under the control of the constitutive TK promoter
 25 was used to normalize the transfections as described above. The transfected cells were grown in the presence 0, 1, 5 or 25 μ M of the non-steroid N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine or the steroid PonA for 48 hours. The cells were harvested, lysed and luciferase reporter activity was measured in the cell lysates. Total fly luciferase relative light units are presented. The number on the top of each bar is the maximum fold induction for that treatment. The analysis was
 30 performed in triplicate and mean luciferase counts [total relative light units (RLU)] were determined as described above.

As shown in the Figures 4-7, CfEcR-CDEF performs better than any other CfEcR truncation. In particular, Gal4CfEcR-CDEF showed better induction than Gal4CfEcR-DEF using VP16LmUSP-EF. The EF domain of CfEcR in combination with LmUSP-DEF showed fairly good induced levels with very
 35 low uninduced levels. Most of EcR-EF domains described in patents and publications include D, E, and F domains (about 300 amino acids). This particular truncation includes only 230 amino acids and may rely on the D domain of LmUSP for heterodimerization.

Of all the truncations of LmUSP tested, Applicants' results show that the VP16LmUSP-EF hybrid receptor polypeptide was the best partner for Gal4CfEcR-based hybrid polypeptides, with GAL4CfEcRCDEF/VP16LmUSP-EF (switch 1.17) performing better than any other receptor combination and more sensitive to non-steroids than steroids (Figures 6 and 7). In general, the CfEcR/LmUSP-based switch was more sensitive to the non-steroid N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine than to the steroid PonA. Thus, the EF domain of LmUSP is sufficient and performs better than DEF domains of this receptor in partnership with CfEcR constructs.

Applicants' results show that the magnitude and fold induction of MmRXR α and LmUSP are similar but LmUSP improves sensitivity to ligand by at least 10 fold. Thus, the EcR/invertebrate system is an improvement over the EcR/vertebrate system.

EXAMPLE 4

This Example describes Applicants' further analysis of gene expression cassettes encoding truncated EcR or RXR receptor polypeptides that affect either ligand binding activity or ligand sensitivity, or both. Briefly, eleven different combinations of two-hybrid receptor pairs, constructed as described in Example 1, were further analyzed in a single experiment in NIH3T3 cells. These eleven receptor pair combinations and their corresponding sample numbers are depicted in Table 1.

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Table 1

CfEcR + MmRXR α /LmUSP Truncation Receptor Combinations in NIH3T3 Cells

Figure 8 X-Axis Sample No.	CfEcR Polypeptide Construct	MmRXR α or LmUSP Polypeptide Construct
Samples 1 and 2	GAL4CfEcR-CDEF	VP16MmRXR α -A/BCDEF
Samples 3 and 4	GAL4CfEcR-CDEF	VP16MmRXR α -DEF
Samples 5 and 6	GAL4CfEcR-CDEF	VP16MmRXR α -EF
Samples 7 and 8	GAL4CfEcR-DEF	VP16MmRXR α -A/BCDEF
Samples 9 and 10	GAL4CfEcR-DEF	VP16MmRXR α -DEF
Samples 11 and 12	GAL4CfEcR-DEF	VP16MmRXR α -EF
Samples 13 and 14	GAL4:CfEcR-CDEF	VP16:LmUSP-DEF
Samples 15 and 16	GAL4:CfEcR-CDEF	VP16:LmUSP-EF
Samples 17 and 18	GAL4:CfEcR-DEF	VP16:LmUSP-DEF
Samples 19 and 20	GAL4:CfEcR-DEF	VP16:LmUSP-EF
Samples 21 and 22	GAL4:CfEcR-EF	VP16:LmUSP-DEF

The above receptor construct pairs, along with the reporter plasmid pFRLuc were constructed as described above and transfected into NIH3T3 cells as described above. The eleven CfEcR truncation receptor combinations were duplicated into two groups and treated with either steroid (odd numbers on x-axis of Figure 8) or non-steroid (even numbers on x-axis of Figure 8). In particular, the cells were cultured in media containing 0, 1, 5 or 25 uM PonA (steroid) or N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine (non-steroid) ligand. The reporter gene activity was measured

and total RLU are shown. The number on top of each bar is the maximum fold induction for that treatment and is the mean of three replicates.

As shown in Figure 8, the CfEcR-CDEF/LmUSP-EF receptor combination (columns 15 and 16) was the best format both in terms of total RLU and fold induction. This result is consistent with

- 5 Applicants' results presented above in Example 3. These eleven receptor pair combinations were also assayed in a human lung carcinoma cell line A549 (ATCC) and similar results were observed (data not shown).

EXAMPLE 5

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This Example describes Applicants' analysis of additional invertebrate retinoid X receptor homologs for use within the EcR/invertebrate RXR-based inducible gene expression system of the present invention. Briefly, two-hybrid receptor gene switches were constructed as described in Example 1 comprising a GAL4/CfEcR-DEF gene expression cassette and VP16AmaRXR1-EF or a
15 VP16AmaRXR2-EF. These AmaRXR1- and AmaRXR2-based gene switches (switches 1.21 and 1.22 of Example 1) were compared to GAL4/CfEcR-DEF gene switches comprising VP16MmRXR α -EF (switch 1.6), VP16LmUSP-EF (switch 1.18), VP16DmUSP-EF (switch 1.29), and VP16CfUSP-EF (switch 1.30) along with pFRLuc in NIH3T3 cells.

- The above receptor construct pairs, along with the reporter plasmid pFRLuc were constructed as
20 described above and transfected into NIH3T3 cells as described above. The transactivation potential of these six CfEcR-DEF receptor-based gene switches were determined in the transfected cells in the presence of 0, 0.2, 1, or 10 μ M PonA (steroid) or 0, 0.4, 0.2, 1, or 10 μ M non-steroid ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine. The reporter gene activity was measured and total RLU are shown. The number on top of each bar is the maximum fold induction for
25 that treatment and is the mean of three replicates.

- As shown in Figure 9, both AmaRXR1-EF and AmaRXR2-EF based switches performed better than the vertebrate MmRXR α -EF based switch, demonstrating that these non-dipteran, non-lepidopteran invertebrate RXR homologs can also function in the EcR/invertebrate RXR-based inducible gene expression system of the present invention. Thus, based upon Applicants' surprising discovery that an
30 invertebrate RXR (LmUSP) can substitute for a vertebrate RXR and the findings borne out in this Example regarding additional invertebrate species RXRs, one of ordinary skill in the art is able to predict that other invertebrate species, non-dipteran and non-lepidopteran RXR homologs will work in Applicant's gene expression system.

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EXAMPLE 6

This Example describes the construction of host cells comprising the EcR/invertebrate RXR-

based gene expression modulation system according to the invention. To make stable cells expressing GAL4:CfEcR-DEF/VP16:LmUSP-EF (switch 1.18, prepared as described in Example 1), Applicants transfected the gene expression cassettes encoding the hybrid GAL4:CfEcR-DEF and VP16:LmUSP-EF polypeptides into Chinese hamster ovary CHO cells comprising a stably transfected reporter plasmid pFRLuc. Briefly, CHO cells were harvested when they reach 60-80% confluency and plated in 6- or 12- or 24- well plates at 250,000, 100,000, or 50,000 cells in 2.5, 1.0, or 0.5 ml of growth medium containing 10% Fetal bovine serum respectively. The next day, the cells were rinsed with growth medium and transfected for four hours. LipofectAMINE™ 2000 (Life Technologies Inc.) was found to be the best transfection reagent for these cells. For 12- well plates, 4 µl of LipofectAMINE™ 2000 was mixed with 100 µl of growth medium. 1.0 µg of reporter construct and 0.25 µg of each receptor construct GAL4:CfEcR-DEF and VP16:LmUSP-EF were added to the transfection mix. A second reporter construct was added (0.1 µg/transfection mix) and comprised a *Renilla* luciferase gene operably linked and placed under the control of a thymidine kinase (TK) constitutive promoter and was used for normalization. The contents of the transfection mix were mixed in a vortex mixer and let stand at room temperature for 30 min. At the end of incubation, the transfection mix was added to the cells maintained in 400 µl growth medium. The cells were maintained at 37°C and 5% CO₂ for four hours. At the end of incubation, 500 µl of growth medium containing 20% FBS and either DMSO (control) or a DMSO solution of appropriate ligands were added and the cells were maintained at 37 °C and 5% CO₂ for 24-48 hr. The cells were harvested and reporter activity was assayed. The same procedure was followed for 6 and 24 well plates as well except all the reagents were doubled for 6 well plates and reduced to half for 24-well plates.

The transfected CHO cells were grown in the presence of 0, 1, 5, or 25 µM PonA steroid ligand or GSTM-E non-steroid ligand for 48 hours. The cells were harvested, lysed and the reporter activity was measured. Total fly luciferase relative light units (RLU) are presented. The numbers on the top of the bars correspond to the maximum fold induction for each treatment. Bulk populations of cells were selected for resistance to the antibiotic neomycin (VP16:LmUSP/VP16:RXR constructs have the neomycin resistance gene incorporated). Several clones from each population were isolated by end point dilution. Three clones of stably transfected GAL4:CfEcR-DEF/VP16:LmUSP-EF cells were analyzed (see Figure 10, clone 1A2; the data related to the two other clones are not shown).

Of the three clones analyzed, the GAL4:CfEcR-DEF/VP16:LmUSP-EF stable clone 1A2 exhibited the highest fold induction, 162 fold, in the presence of non-steroidal ligand and 42 fold induction in the presence of steroid PonA (see Figure 10).

EXAMPLE 7

This Example describes the development of another embodiment of the EcR/invertebrate RXR gene expression modulation system of the invention. Specifically, Applicants have constructed LexA

DNA binding domain (DBD) based-EcR/invertebrate RXR gene switches for use in the gene expression modulation system of the invention. This embodiment can be useful as an alternate switch for a GAL4DBD-based switch and can also be used in multiple switch formats. While the LexA DBD has been used in yeast and plant expression systems, Applicants are not aware of its use in mammalian applications.

Briefly, a gene expression cassette comprising the LexA DNA binding domain (SEQ ID NO: 35) fused to CfEcR-CDEF domains (SEQ ID NO: 45) was prepared as described in Example 1. The LexA:CfEcR-CDEF gene expression cassette, along with a VP16:MmRXR α -EF, a VP16:CfUSP-EF, a VP16:DmUSP-EF, or a VP16:LmUSP-EF gene expression cassette, and a reporter construct (8opFRLuc) comprising an 8XLexA operator (4 copies of LexA response element; SEQ ID NO: 42), a minimal promoter (synthetic E1b minimal promoter SEQ ID NO: 48), and a luciferase gene (SEQ ID NO: 49) were transfected into mouse NIH3T3 cells. The transfected cells were cultured in the presence of 0, 0.1, 1, 5, 10, and 50 μ M GSTM-E non-steroidal ligand or PonA steroid ligand for 48 hours as described above. The cells were harvested, lysed and reporter activity was measured and total relative light units (RLU) are presented in Figure 11. The number on the top of each bar corresponds to the maximum fold induction of each treatment.

The LexA:CfEcR-DEF construct functioned well in these mammalian cells with all partners examined (see Figure 11). The fold induction is comparable to what was observed with Applicants' GAL4 system (see Figure 4). The 8opFRLuc reporter (control) showed very little activity in these cells. The results presented in Figure 11 show that the LexA DNA binding domain functions well in Applicants' two-hybrid system, demonstrating that the DNA binding domain is portable in these gene expression cassettes.

EXAMPLE 8

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This Example describes the development of another embodiment of the EcR/invertebrate RXR gene expression modulation system of the invention. Specifically, Applicants have constructed gene expression cassettes for use in the gene modulation system of the invention comprising a B42 acidic activator domain as a transactivation domain. The B42 acidic activator domain ("B42AD"; see Gyuris *et al.*, (1993) Cell 75: 791-803) works well as a transactivator in yeast and as Applicants have now shown, works well in mammalian cells. The B42 acidic activator domain may be used in the gene expression cassettes of the present invention as an alternative to VP16 transactivation domain.

Briefly, Applicants have constructed a gene expression cassette comprising a polynucleotide encoding a B42AD (SEQ ID NO: 39) fused to a polynucleotide encoding LmUSP-EF domains (SEQ ID NO: 9) as described in Example 1. This B42AD:LmUSP-EF gene expression cassette was evaluated in mouse NIH3T3 cells in partnership with either a GAL4:CfEcR-CDEF or a LexA:CfEcR-CDEF gene expression cassette and compared to a VP16:LmUSP-EF-based switch. All gene expression cassettes

were prepared as described in Example 1. The appropriate reporter constructs were transfected into NIH3T3 cells. The transfected cells were cultured in the presence of 0, 0.1, 1, 5, 10, and 50 μM GSTM-E non-steroidal ligand for 48 hours as described above. Reporter activity is plotted as total RLU (see Figure 12). The numbers on the top of the bars correspond to the maximum fold induction observed for that combination.

The results show that the B42 acidic activation domain works as well as the VP16 transactivation domain in Applicants' two-hybrid system, demonstrating that the transactivation domain is also portable in these gene expression cassettes.

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EXAMPLE 9

This Example demonstrates the effect of introduction of a second ligand into the host cell comprising an EcR/invertebrate RXR-based inducible gene expression modulation system of the invention. In particular, Applicants have determined the effect of 9-cis-retinoic acid on the transactivation potential of the GAL4CfEcR-DEF/VP16LmUSP-EF (switch 1.18) gene switch along with pFRLuc in NIH3T3 cells in the presence of non-steroid (GSE) for 48 hours.

Briefly, GAL4CfEcR-DEF, pFRLuc and VP16LmUSP-EF were transfected into NIH3T3 cells and the transfected cells were treated with 0, 0.04, 0.2, 1, 5 and 25 μM non-steroidal ligand (GSE) and 0, 1, 5 and 25 μM 9-Cis-retinoic acid (Sigma Chemical Company). The reporter activity was measured at 48 hours after adding ligands.

As shown in Figure 13, the presence of retinoic acid increased the sensitivity of CfEcR-DEF to non-steroidal ligand. At a non-steroid ligand concentration of 1 μM or less, there is very little induction in the absence of 9-Cis-retinoic acid, but when 1 μM 9-Cis-retinoic acid is added in addition to non-steroid, induction is greatly increased.

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WE CLAIM:

1. A gene expression modulation system comprising:
 - a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising:
 - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
 - ii) an ecdysone receptor ligand binding domain; and
 - b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:
 - i) a transactivation domain; and
 - ii) an invertebrate retinoid X receptor ligand binding domain.
2. The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:
 - i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide binds;
 - ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
 - iii) a gene whose expression is to be modulated.
3. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain (LBD) of the first hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR ("CtEcR") LBD, a silk moth *Bombyx mori* EcR ("BmEcR") LBD, a fruit fly *Drosophila melanogaster* EcR ("DmEcR") LBD, a mosquito *Aedes aegypti* EcR ("AaEcR") LBD, a blowfly *Lucilia capitata* ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratitis capitata* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Myzus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bemisia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.
4. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.
5. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

6. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

7. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

8. The gene expression modulation system according to claim 1, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an ecdysone receptor ligand binding domain.

9. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an invertebrate retinoid X receptor ligand binding domain.

10. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

11. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

12. A gene expression modulation system comprising:
a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising:
i) a DNA-binding domain that recognizes a response element associated with a

gene whose expression is to be modulated; and

ii) an invertebrate retinoid X receptor ligand binding domain; and

b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:

5 i) a transactivation domain; and

ii) an ecdysone receptor ligand binding domain.

13. The gene expression modulation system according to claim 12, further comprising a third gene expression cassette comprising:

10 i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide binds;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated.

14. The gene expression modulation system according to claim 12, wherein the invertebrate
15 retinoid X receptor ligand binding domain of the first hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

15. The gene expression modulation system according to claim 12, wherein the invertebrate
20 retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

16. The gene expression modulation system according to claim 12, wherein the ecdysone
25 receptor ligand binding domain of the second hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR ("CtEcR") LBD, a silk moth *Bombyx mori* EcR ("BmEcR") LBD, a fruit fly *Drosophila melanogaster* EcR ("DmEcR") LBD, a mosquito *Aedes aegypti* EcR ("AaEcR") LBD, a
30 blowfly *Lucilia capitata* ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratitidis capitata* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Myzus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bamecia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.

35 17. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:

53 and SEQ ID NO: 45.

18. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

5 19. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an invertebrate retinoid X receptor ligand binding domain.

20. The gene expression modulation system according to claim 12, wherein the first gene
1 0 expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) or a LexA DBD (SEQ ID NO: 35) and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11,
1 5 SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

21. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4
2 0 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

22. The gene expression modulation system according to claim 12, wherein the second gene
2 5 expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an ecdysone receptor ligand binding domain.

23. A gene expression cassette comprising a polynucleotide that encodes a hybrid
3 0 polypeptide comprising a DNA-binding domain and an invertebrate retinoid X receptor ligand binding domain, wherein the DNA binding domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

24. The gene expression cassette according to claim 23, wherein the DNA-binding domain is a GAL4 DNA-binding domain or a LexA DNA-binding domain.

25. The gene expression cassette according to claim 23, wherein the gene expression
3 5 cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) and a LexA DBD (SEQ ID NO: 35), and an invertebrate

retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

5 26. The gene expression cassette according to claim 23, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

 27. A gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain and an invertebrate retinoid X receptor ligand binding domain, wherein the transactivation domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

 28. The gene expression cassette according to claim 27, wherein the transactivation domain is a VP16 transactivation domain or a B42 acidic activator transactivation domain.

 29. The gene expression cassette according to claim 27, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

 30. The gene expression cassette according to claim 29, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

 31. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

 32. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces steroid

binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

33. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

5 34. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

35. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances steroid
10 binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

36. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain.

37. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand
15 binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of the truncated invertebrate retinoid X receptor ligand binding domain.

38. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises said truncated invertebrate retinoid X
20 receptor ligand binding domain and a dimerization partner.

39. The isolated polynucleotide according to claim 38, wherein the dimerization partner is an ecdysone receptor polypeptide.

40. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain, wherein the polynucleotide comprises a nucleic acid sequence selected from the group
25 consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

41. An isolated polypeptide encoded by the isolated polynucleotide according to claim 40.

42. An isolated truncated invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID
30 NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

43. A method of modulating the expression of a gene in a host cell comprising the steps of:
a) introducing into the host cell the gene expression modulation system according to claim 1; and

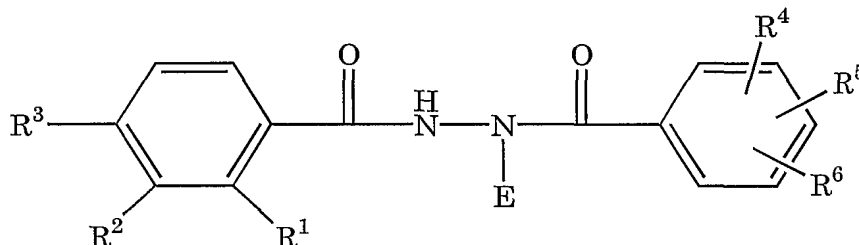
b) introducing into the host cell a ligand;
35 wherein the gene to be modulated is a component of a gene expression cassette comprising:
i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;

ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and

iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

44. The method according to claim 43, wherein the ligand is a compound of the formula:



wherein:

E is a (C₄-C₆)alkyl containing a tertiary carbon or a cyano(C₃-C₅)alkyl containing a tertiary carbon;

R¹ is H, Me, Et, i-Pr, F, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, SCN, or SCHF₂;

R² is H, Me, Et, n-Pr, i-Pr, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe₂, NEt₂, SMe, SEt, SOCF₃, OCF₂CF₂H, COEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, OCF₃, OCHF₂, O-i-Pr, SCN, SCHF₂, SOMe, NH-CN, or joined with R³ and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R³ is H, Et, or joined with R² and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R⁴, R⁵, and R⁶ are independently H, Me, Et, F, Cl, Br, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

45. The method according to claim 43, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

46. A method of modulating the expression of a gene in a host cell comprising the steps of:

- introducing into the host cell the gene expression modulation system of claim 11; and
- introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

- a response element recognized by the DNA binding domain from the first hybrid polypeptide;
- a promoter that is activated by the transactivation domain of the

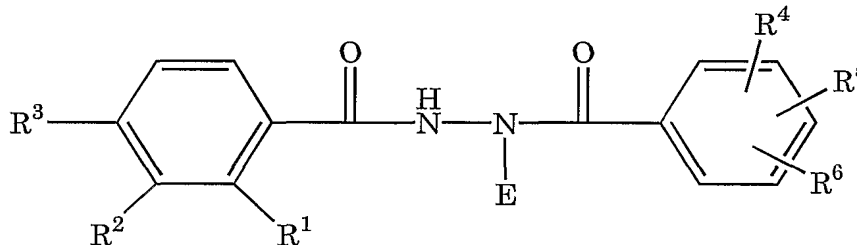
second hybrid polypeptide; and

iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

47. The method according to claim 46, wherein the ligand is a compound of the

5 formula:



wherein:

E is a (C₄-C₆)alkyl containing a tertiary carbon or a cyano(C₃-C₅)alkyl containing a tertiary carbon;

10 R¹ is H, Me, Et, i-Pr, F, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, SCN, or SCHF₂;

R² is H, Me, Et, n-Pr, i-Pr, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe, CH₂CN, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe₂, NEt₂, SMe, SEt, SOCF₃, OCF₂CF₂H, COEt, cyclopropyl, CF₂CF₃, CH=CHCN, allyl, azido, OCF₃,
15 OCHF₂, O-i-Pr, SCN, SCHF₂, SOMe, NH-CN, or joined with R³ and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R³ is H, Et, or joined with R² and the phenyl carbons to which R² and R³ are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a
20 dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R⁴, R⁵, and R⁶ are independently H, Me, Et, F, Cl, Br, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CN, C^oCH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

48. The method according to claim 46, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

25 49. An isolated host cell comprising the gene expression modulation system according to claim 1.

50. The isolated host cell according to claim 49, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

51. The isolated host cell according to claim 50, wherein the mammalian cell is a murine cell
30 or a human cell.

52. An isolated host cell comprising the gene expression modulation system according to claim 12.

53. The isolated host cell according to claim 52, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

54. The isolated host cell according to claim 53, wherein the mammalian cell is a murine cell or a human cell.

5 55. A non-human organism comprising the host cell of claim 52.

56. The non-human organism according to claim 55, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.

57. The non-human organism according to claim 56, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a
10 monkey, and a chimpanzee.

58. A non-human organism comprising the host cell of claim 52.

59. The non-human organism according to claim 58, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.

60. The non-human organism according to claim 59, wherein the mammal is selected from
15 the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.

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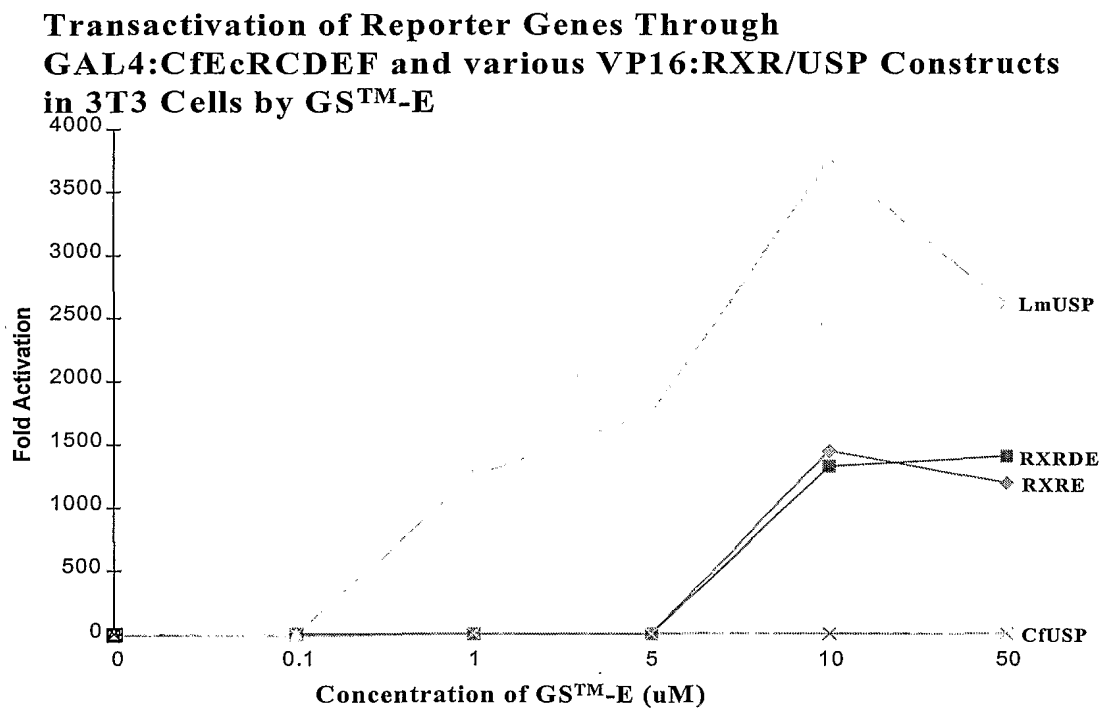


Figure 1

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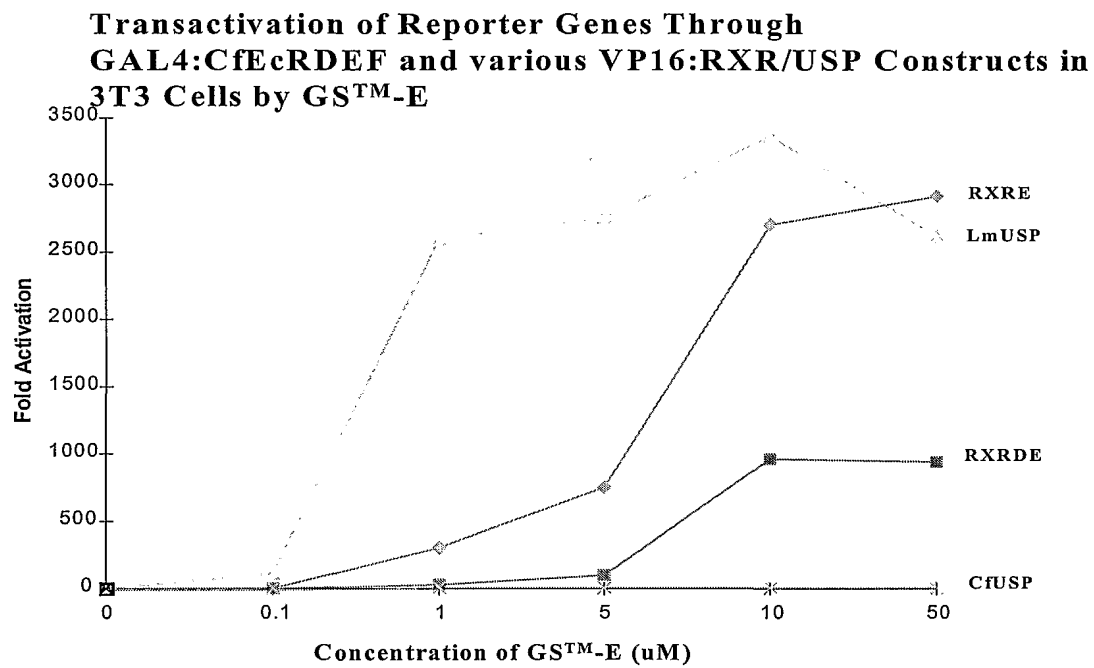


Figure 2

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HsRXRgEF	GHEDMPVERILEAELAVEPKTESYGDMN-----MENSTNDPVTNICHAAADKQFLTLVEWA	55
MmRXRgEF	SHEDMPVERILEAELAVEPKTESYGDMN-----VENSTNDPVTNICHAAADKQFLTLVEWA	55
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		B6
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		B8A1 B9
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Figure 3A

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AmaRXR2EF GLRNATRVEALREKVYAAL E E H C R R H H P D O P G R F G K L L L R L P A L R S I G L K C L E H L F F F F K L 210
      H9      H10      H11

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Figure 3B

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Analysis of CfEcR Truncations with MmRXRDE in 3T3 Cells

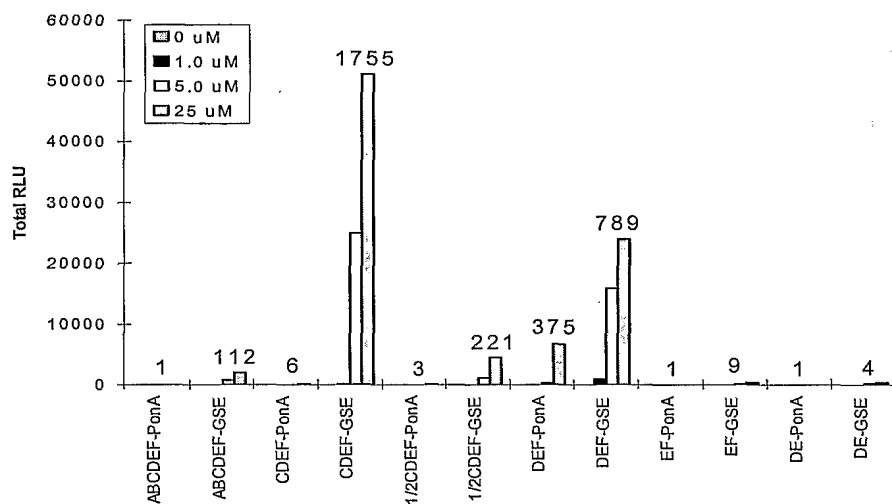


Figure 4

Analysis of CfEcR Truncations with MmRXRE in 3T3 Cells

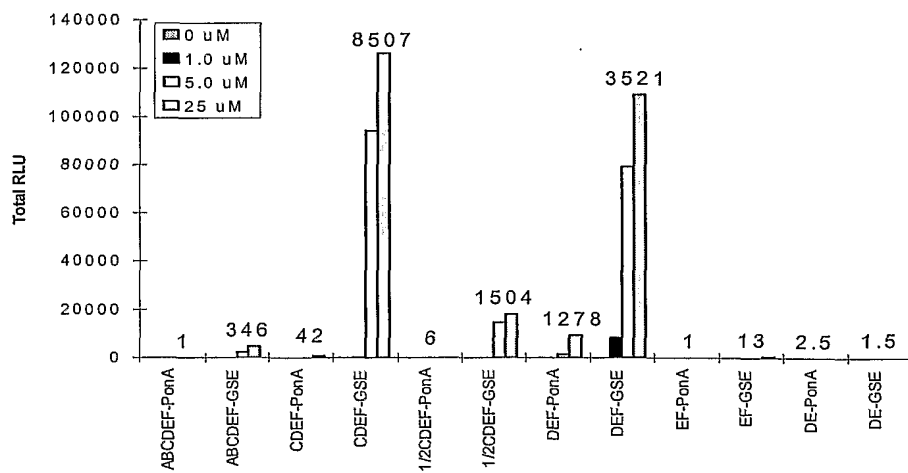


Figure 5

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Analysis of CfEcR Truncations with Lm USPDE in 3T3 Cells

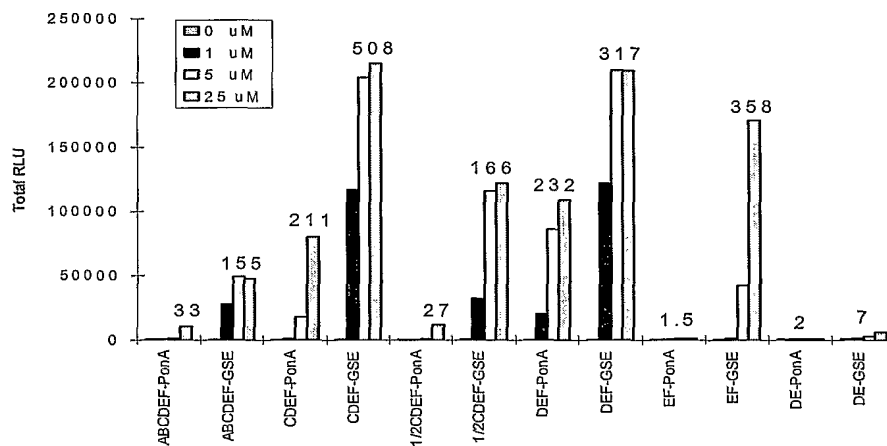


Figure 6

Analysis of CfEcR Truncations with LmUSPE in 3T3 Cells

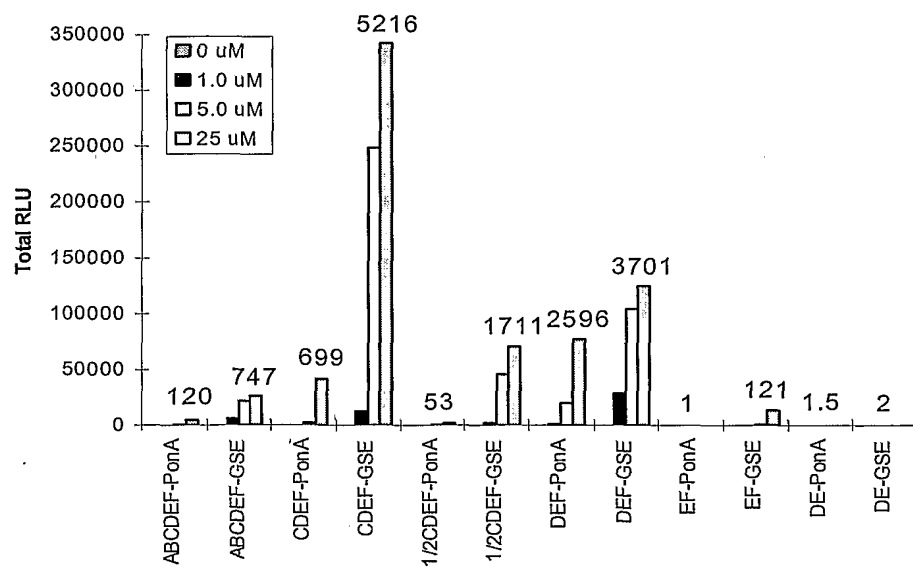


Figure 7

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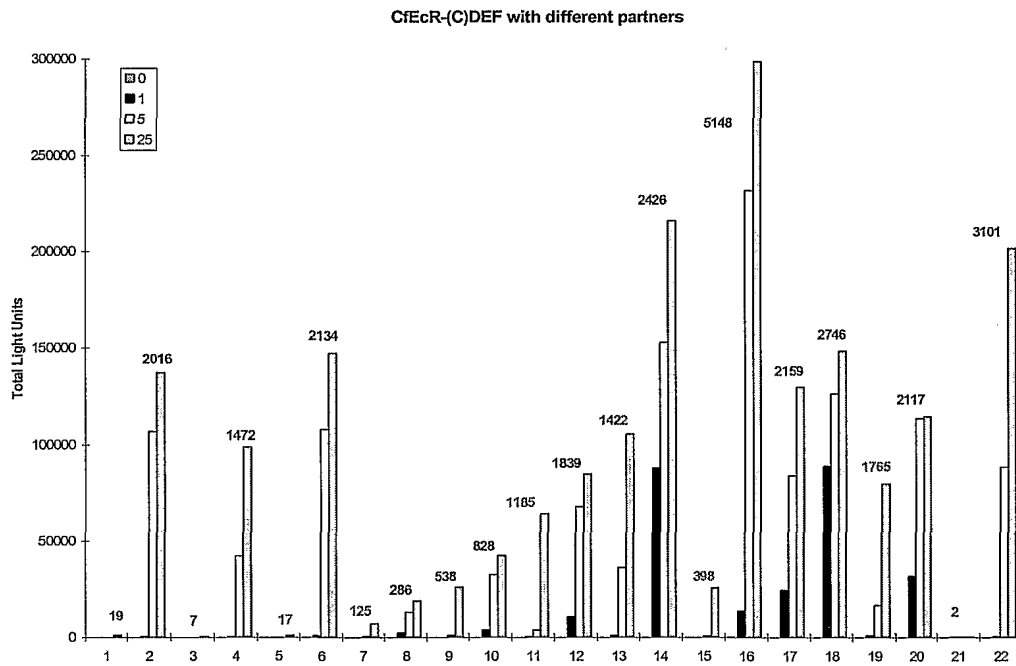


Figure 8

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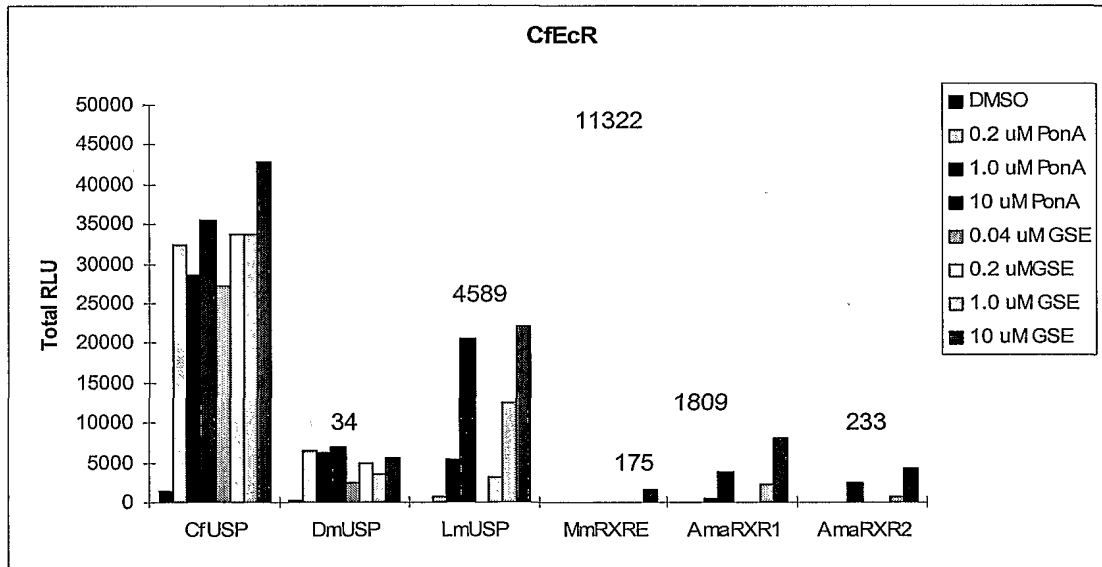


Figure 9

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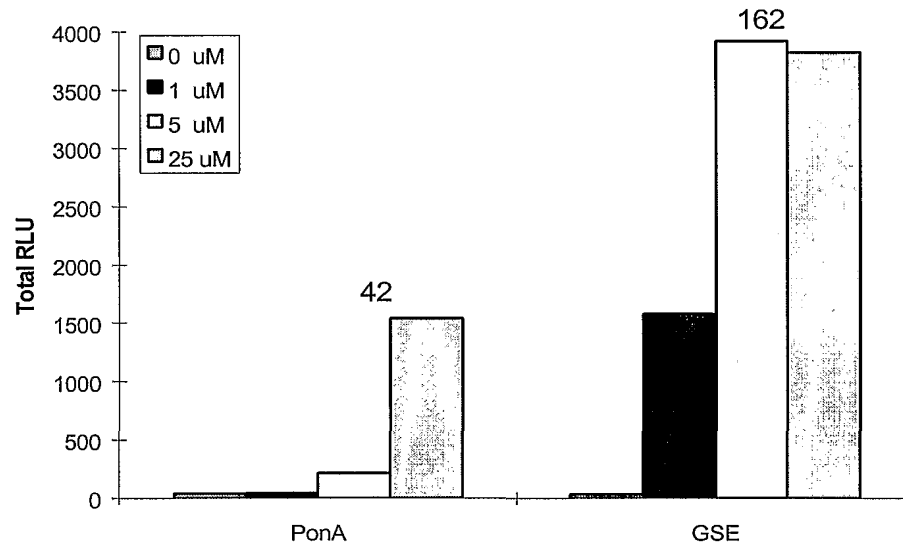
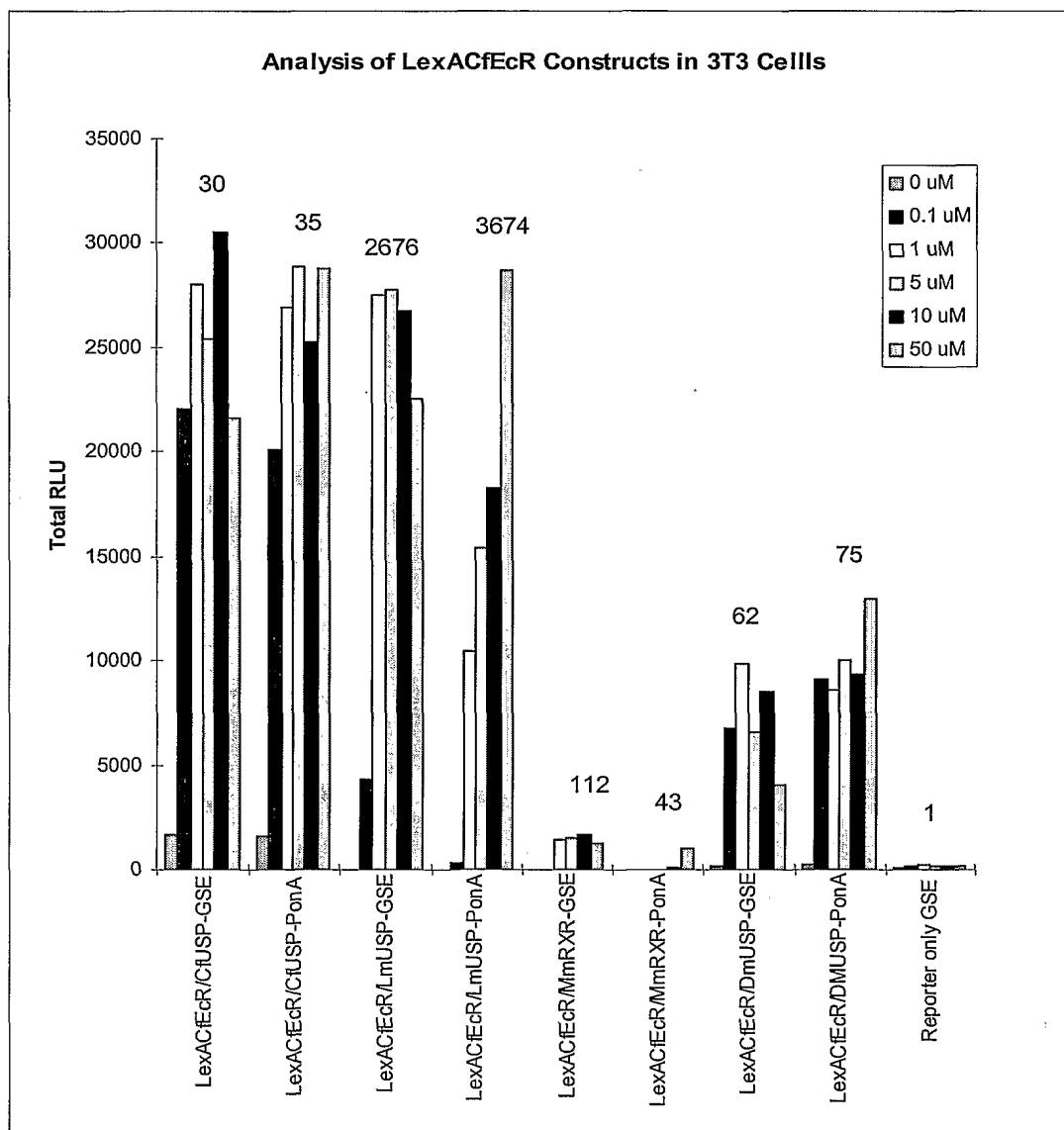


Figure 10

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**Figure 11**

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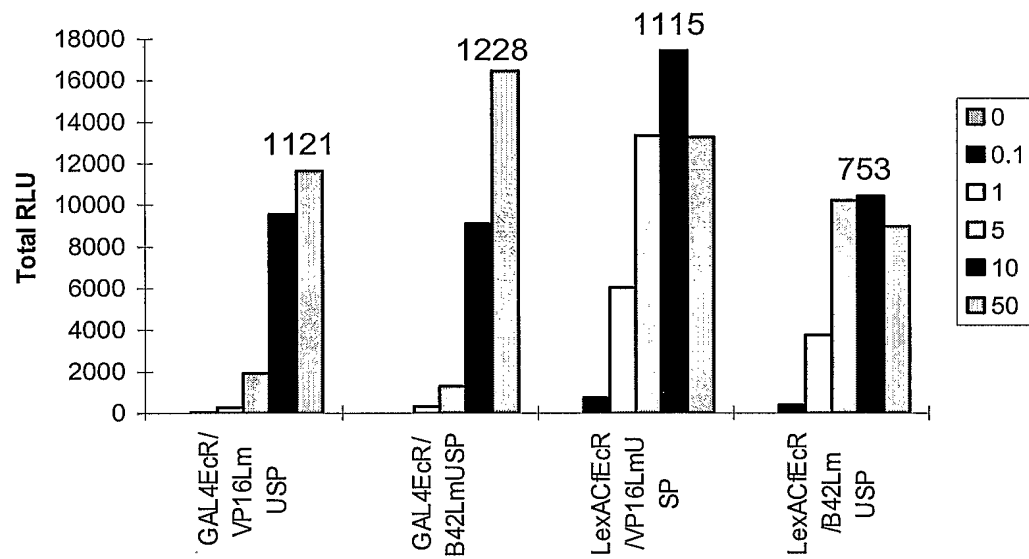


Figure 12

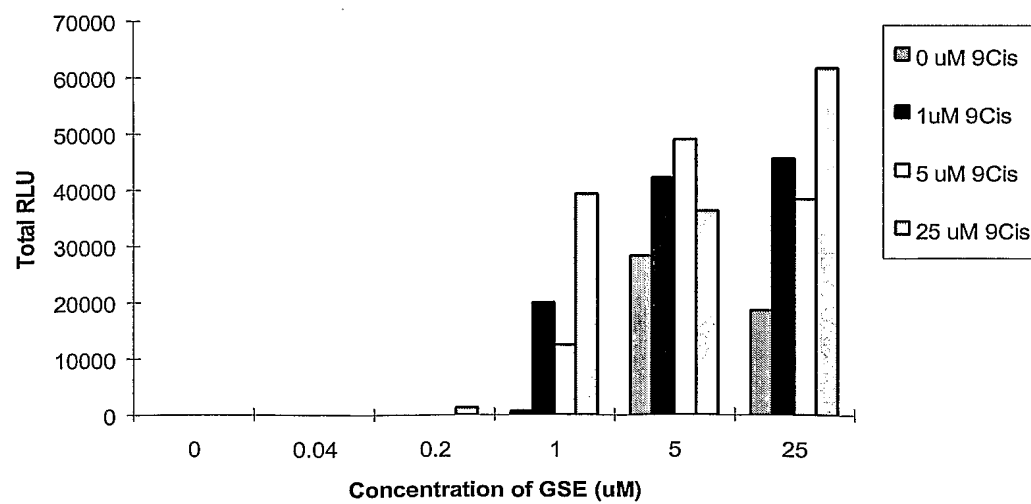


Figure 13

SEQUENCE LISTING

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 Palli, Subba R.
 Kapitskaya, Marianna Z.

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 inducible gene expression system

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 ttctacgcaa agctgctctc gatcctcacc gagctgcgta cgctgggcaa ccagaacgcc 900
 gagatgtgtt totcactaaa gctcaaaaac cgcaaactgc ccaagttcct cgaggagatc 960
 tgggacggtt 969

<210> 5
 <211> 244
 <212> PRT
 <213> Choristoneura fumiferana

<400> 5

Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile
 1 5 10 15

Thr Gln Thr Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr
 20 25 30

Pro Phe Arg Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile
 35 40 45

Val Glu Phe Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro
 50 55 60

Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu
 65 70 75 80

Arg Val Ala Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala
 85 90 95

Asn Asn Gln Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala
 100 105 110

Tyr Val Ile Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met
 115 120 125

Ala Leu Asp Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe
 130 135 140

Ser Asp Arg Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln
 145 150 155 160

Arg Tyr Tyr Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser
 165 170 175

Gly Ser Ala Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu
 180 185 190

Ser Glu Leu Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser
 195 200 205

Leu Lys Leu Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp

210 215 220
 Asp Val Ala Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser
 225 230 235 240

 Pro Thr Asn Leu

 <210> 6
 <211> 445
 <212> PRT
 <213> Drosophila melanogaster

 <400> 6

 Tyr Glu Gln Pro Ser Glu Glu Asp Leu Arg Arg Ile Met Ser Gln Pro
 1 5 10 15

 Asp Glu Asn Glu Ser Gln Thr Asp Val Ser Phe Arg His Ile Thr Glu
 20 25 30

 Ile Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly Leu
 35 40 45

 Pro Ala Phe Thr Lys Ile Pro Gln Glu Asp Gln Ile Thr Leu Leu Lys
 50 55 60

 Ala Cys Ser Ser Glu Val Met Met Leu Arg Met Ala Arg Arg Tyr Asp
 65 70 75 80

 His Ser Ser Asp Ser Ile Phe Phe Ala Asn Asn Arg Ser Tyr Thr Arg
 85 90 95

 Asp Ser Tyr Lys Met Ala Gly Met Ala Asp Asn Ile Glu Asp Leu Leu
 100 105 110

 His Phe Cys Arg Gln Met Phe Ser Met Lys Val Asp Asn Val Glu Tyr
 115 120 125

 Ala Leu Leu Thr Ala Ile Val Ile Phe Ser Asp Arg Pro Gly Leu Glu
 130 135 140

 Lys Ala Gln Leu Val Glu Ala Ile Gln Ser Tyr Tyr Ile Asp Thr Leu
 145 150 155 160

 Arg Ile Tyr Ile Leu Asn Arg His Cys Gly Asp Ser Met Ser Leu Val
 165 170 175

Phe Tyr Ala Lys Leu Leu Ser Ile Leu Thr Glu Leu Arg Thr Leu Gly
 180 185 190

Asn Gln Asn Ala Glu Met Cys Phe Ser Leu Lys Leu Lys Asn Arg Lys
 195 200 205

Leu Pro Lys Phe Leu Glu Glu Ile Trp Asp Val His Ala Ile Pro Pro
 210 215 220

Ser Val Gln Ser His Leu Gln Ile Thr Gln Glu Glu Asn Glu Arg Leu
 225 230 235 240

Glu Arg Ala Glu Arg Met Arg Ala Ser Val Gly Gly Ala Ile Thr Ala
 245 250 255

Gly Ile Asp Cys Asp Ser Ala Ser Thr Ser Ala Ala Ala Ala Ala Ala
 260 265 270

Gln His Gln Pro Gln Pro Gln Pro Gln Pro Gln Pro Ser Ser Leu Thr
 275 280 285

Gln Asn Asp Ser Gln His Gln Thr Gln Pro Gln Leu Gln Pro Gln Leu
 290 295 300

Pro Pro Gln Leu Gln Gly Gln Leu Gln Pro Gln Leu Gln Pro Gln Leu
 305 310 315 320

Gln Thr Gln Leu Gln Pro Gln Ile Gln Pro Gln Pro Gln Leu Leu Pro
 325 330 335

Val Ser Ala Pro Val Pro Ala Ser Val Thr Ala Pro Gly Ser Leu Ser
 340 345 350

Ala Val Ser Thr Ser Ser Glu Tyr Met Gly Gly Ser Ala Ala Ile Gly
 355 360 365

Pro Ile Thr Pro Ala Thr Thr Ser Ser Ile Thr Ala Ala Val Thr Ala
 370 375 380

Ser Ser Thr Thr Ser Ala Val Pro Met Gly Asn Gly Val Gly Val Gly
 385 390 395 400

Val Gly Val Gly Gly Asn Val Ser Met Tyr Ala Asn Ala Gln Thr Ala
 405 410 415

Met Ala Leu Met Gly Val Ala Leu His Ser His Gln Glu Gln Leu Ile
 420 425 430

Gly Gly Val Ala Val Lys Ser Glu His Ser Thr Thr Ala
 435 440 445

<210> 7

<211> 320

<212> PRT

<213> Choristoneura fumiferana

<400> 7

Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys Arg Lys Glu
 1 5 10 15

Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr
 20 25 30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro
 35 40 45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys
 50 55 60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn
 65 70 75 80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu
 85 90 95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln
 100 105 110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr
 115 120 125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly
 130 135 140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu
 145 150 155 160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr
 165 170 175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr
 180 185 190

Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu
 195 200 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His
 210 215 220

Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu
 225 230 235 240

Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr
 245 250 255

Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser
 260 265 270

Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu
 275 280 285

Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg
 290 295 300

Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser
 305 310 315 320

<210> 8
 <211> 323
 <212> PRT
 <213> *Drosophila melanogaster*

<400> 8

Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg
 1 5 10 15

Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser
 20 25 30

Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp
 35 40 45

Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln
 50 55 60

His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln
 65 70 75 80

Ala	Arg	Asn	Ile	Pro	Ser	Leu	Thr	Tyr	Asn	Gln	Leu	Ala	Val	Ile	Tyr	85	90	95	
Lys	Leu	Ile	Trp	Tyr	Gln	Asp	Gly	Tyr	Glu	Gln	Pro	Ser	Glu	Glu	Asp	100	105	110	
Leu	Arg	Arg	Ile	Met	Ser	Gln	Pro	Asp	Glu	Asn	Glu	Ser	Gln	Thr	Asp	115	120	125	
Val	Ser	Phe	Arg	His	Ile	Thr	Glu	Ile	Thr	Ile	Leu	Thr	Val	Gln	Leu	130	135	140	
Ile	Val	Glu	Phe	Ala	Lys	Gly	Leu	Pro	Ala	Phe	Thr	Lys	Ile	Pro	Gln	145	150	155	160
Glu	Asp	Gln	Ile	Thr	Leu	Leu	Lys	Ala	Cys	Ser	Ser	Glu	Val	Met	Met	165	170	175	
Leu	Arg	Met	Ala	Arg	Arg	Tyr	Asp	His	Ser	Ser	Asp	Ser	Ile	Phe	Phe	180	185	190	
Ala	Asn	Asn	Arg	Ser	Tyr	Thr	Arg	Asp	Ser	Tyr	Lys	Met	Ala	Gly	Met	195	200	205	
Ala	Asp	Asn	Ile	Glu	Asp	Leu	Leu	His	Phe	Cys	Arg	Gln	Met	Phe	Ser	210	215	220	
Met	Lys	Val	Asp	Asn	Val	Glu	Tyr	Ala	Leu	Leu	Thr	Ala	Ile	Val	Ile	225	230	235	240
Phe	Ser	Asp	Arg	Pro	Gly	Leu	Glu	Lys	Ala	Gln	Leu	Val	Glu	Ala	Ile	245	250	255	
Gln	Ser	Tyr	Tyr	Ile	Asp	Thr	Leu	Arg	Ile	Tyr	Ile	Leu	Asn	Arg	His	260	265	270	
Cys	Gly	Asp	Ser	Met	Ser	Leu	Val	Phe	Tyr	Ala	Lys	Leu	Leu	Ser	Ile	275	280	285	
Leu	Thr	Glu	Leu	Arg	Thr	Leu	Gly	Asn	Gln	Asn	Ala	Glu	Met	Cys	Phe	290	295	300	
Ser	Leu	Lys	Leu	Lys	Asn	Arg	Lys	Leu	Pro	Lys	Phe	Leu	Glu	Glu	Ile	305	310	315	320
Trp	Asp	Val																	

<210> 9
 <211> 635
 <212> DNA
 <213> *Locusta migratoria*

<400> 9
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 cagaaaacca agtggaatat gagctgggtgg agtgggctaa acacatcccg cacttcacat 120
 ccctacctct ggaggaccag gttctcctcc tcagagcagg ttggaatgaa ctgctaattg 180
 cagcattttc acatcgatct gtagatgtta aagatggcat agtacttgcc actgggtctca 240
 cagtgcacgc aaattctgcc catcaagctg gagtgggcac aatatttgac agagttttga 300
 cagaactggt agcaaagatg agagaaatga aaatggataa aactgaactt ggctgcttgc 360
 gatctgttat tcttttcaat ccagaggtga ggggtttgaa atccgccag gaagttgaac 420
 ttctacgtga aaaagtatat gccgctttgg aagaatatac tagaacaaca catcccgatg 480
 aaccaggaag atttgcaaaa cttttgcttc gtctgccttc tttacgttcc ataggcctta 540
 agtggttgga gcatttggtt ttctttcgcc ttattggaga tgttccaatt gatacgttcc 600
 tgatggagat gcttgaatca ccttctgatt cataa 635

<210> 10
 <211> 687
 <212> DNA
 <213> *Amblyomma americanum*

<400> 10
 cctcctgaga tgcctctgga ggcatactg gaggcagagc tgcggggtga gtcacagacg 60
 gggaccctct cggaaagcgc acagcagcag gatccagtga gcagcatctg ccaagctgca 120
 gaccgacagc tgcaccagct agttcaatgg gccaaagcaca ttccacattt tgaagagctt 180
 ccccttgagg accgcatggt gttgctcaag gctggctgga acgagctgct cattgctgct 240
 ttctcccacc gttctgttga cgtgcgtgat ggcattgtgc tcgctacagg tcttgtggtg 300
 cagcggcata gtgctcatgg ggctggcggt ggggccatat ttgatagggt tctcactgaa 360
 ctggtagcaa agatgcgtga gatgaagatg gaccgcactg agcttggatg cctgcttgc 420
 gtggtacttt ttaatcctga ggccaagggg ctgcggacct gcccaagtgg aggcctgag 480
 ggagaaagtg tatctgcctt ggaagagcac tgccggcagc agtaccaga ccagcctggg 540
 cgctttgcca agctgctgct gcggttgcca gctctgcgca gtattggcct caagtgcctc 600
 gaacatctct ttttcttcaa gctcatcggg gacacgcca tcgacaactt tcttcttcc 660
 atgctggagg cccctctga ccctaa 687

<210> 11
 <211> 693
 <212> DNA
 <213> Amblyomma americanum

<400> 11
 tctccggaca tgccactcga acgcattctc gaagccgaga tgcgcgtcga gcagccggca 60
 ccgtccgttt tggcgcagac ggccgcacgc ggccgcgacc ccgtcaacag catgtgccag 120
 gctgccccgc cacttcacga gctcgtacag tgggccccgc gaattccgca cttcgaagag 180
 cttcccatcg aggatcgcac cgcgctgctc aaagccggct ggaacgaact gcttattgcc 240
 gcctttttcgc accgtttctgt ggccggtgcgc gacggcatcg ttctggccac cgggctggtg 300
 gtgcagcggc acagcgcaca cggcgcaggc gttggcgaca tcttcgaccg cgtactagcc 360
 gagctggtgg ccaagatgcg cgacatgaag atggacaaaa cggagctcgg ctgcctgcgc 420
 gccgtggtgc tcttcaatcc agacgccaa ggtctccgaa acgccaccag agtagaggcg 480
 ctccgcgaga aggtgtatgc ggcgctggag gagcactgcc gtcggcacca cccggaccaa 540
 ccgggtcgct tcggcaagct gctgctgcgg ctgcctgcct tgcgcagcat cgggctcaaa 600
 tgccctcgagc atctgttctt cttcaagctc atcggagaca ctcccataga cagcttctctg 660
 ctcaacatgc tggaggcacc ggcagacccc tag 693

<210> 12
 <211> 801
 <212> DNA
 <213> Celuca pugilator

<400> 12
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 cagccgctgg accaaggggt gaggcttcag gttccactcg cacctcctga tagtgaagag 120
 tgtagcttta ctttaccttt tcatcccgtc agtgaagtat cctgtgctaa ccctctgcag 180
 gatgtggtga gcaacatatg ccaggcagct gacagacatc tgggtgcagct ggtggagtgg 240
 gccaaagcaca tcccacactt cacagacctt cccatagagg accaagtggg attactcaaa 300
 gccgggtgga acgagttgct tattgcctca ttctcacacc gtagcatggg cgtggaggat 360
 ggcatcgtgc tggccacagg gctcgtgatc cacagaagta gtgctcacca ggctggagtg 420
 ggtgccatat ttgatcgtgt cctctctgag ctgggtggcca agatgaagga gatgaagatt 480
 gacaagacag agctgggctg ccttcgctcc atcgtcctgt tcaaccaga tgccaaagga 540
 ctaaactgcg tcaatgatgt ggagatcttg cgtgagaagg tgtatgctgc cctggaggag 600
 tacacacgaa ccacttaccc tgatgaacct ggacgctttg ccaagttgct tctgcgactt 660

cctgcactca ggtctatagg cctgaagtgt cttgagtacc tcttcctggt taagctgatt 720
 ggagacactc ccctggacag ctacttgatg aagatgctcg tagacaaccc aaatacaagc 780
 gtcactcccc ccaccagcta g 801

<210> 13
 <211> 690
 <212> DNA
 <213> Tenebrio molitor

<400> 13
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 ggtggctctg gtgggtgctg agagcaacac gacgggggtga acaacatctg tcaagccact 120
 aacaagcagc tgttccaact ggtgcaatgg gctaagctca tacctcactt tacctcggtg 180
 ccgatgtcgg accagggtgct tttattgagg gcaggatgga atgaattgct catcgccgca 240
 ttctcgcaca gatctatata ggcgaggat gccatcggtc tagccacggg gttgacagtt 300
 aacaaaacgt cggcgacgc cgtgggcgtg ggcaacatct acgaccgct cctctccgag 360
 ctggtgaaca agatgaaaga gatgaagatg gacaagacgg agctgggctg cttgagagcc 420
 atcatctctt acaaccccac gtgtcgcggc atcaagtccg tgcaggaagt ggagatgctg 480
 cgtgagaaaa ttacggcgt gctggaagag tacaccagga ccaccaccc gaacgagccc 540
 ggcaggttcg ccaaactgct tctgcgcctc ccggccctca ggtccatcgg gttgaaatgt 600
 tccgaacacc tctttttctt caagctgata ggtgatgttc caatagacac gttcctgatg 660
 gagatgctgg agtctccggc ggacgcttag 690

<210> 14
 <211> 681
 <212> DNA
 <213> Apis mellifera

<400> 14
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 gagcaacagg gaaattacga gaatgcagtg tcgcacatctt gcaacgccac gaacaaacag 120
 ctgttccagc tggtagcatg ggcgaaacac atcccgcat t tacctcggt gccactggag 180
 gatcaggtag ttctgctcag ggccgggttg aacgagttgc tgatagcctc cttttccac 240
 cgttccatcg acgtgaagga cggatcgtg ctggcgacgg ggatcacctg gcatcggaac 300
 tcggcgacgc aggcggcgt gggcacgata ttgcaccgtg tctctcgga gcttgtctcg 360
 aaaatgcgtg aatgaagat ggacaggaca gagcttggt gtctcagatc tataatactc 420
 ttcaatcccg aggttcgagg actgaaatcc atccaggaag tgaccctgct ccgtgagaag 480
 atctacggcg ccctggaggg ttattgccgc gtagcttggc ccgacgacgc tggaagattc 540

gcgaaattac ttctacgcct gcccgccatc cgctcgatcg gattaaagtg cctcgagtac 600
 ctgtttcttct tcaaaatgat cgggtgacgta ccgatcgacg attttctcgt ggagatgtta 660
 gaatcgcgat cagatcctta g 681

<210> 15
 <211> 516
 <212> DNA
 <213> *Locusta migratoria*

<400> 15
 atccctacct ctggaggacc aggttctcct cctcagagca gggtggaatg aactgctaata 60
 tgcagcattt tcacatcgat ctgtagatgt taaagatggc atagtacttg ccactgggtct 120
 cacagtgcac cgaaattctg cccatcaagc tggagtcggc acaatatttg acagagtttt 180
 gacagaactg gtagcaaaga tgagagaaat gaaaatggat aaaactgaac ttggctgctt 240
 gcgatctgtt attcttttca atccagaggt gaggggtttg aaatccgccc aggaagttga 300
 acttctacgt gaaaaagtat atgccgcttt ggaagaatat actagaacaa cacatcccga 360
 tgaaccagga agatttgcaa aacttttgct tcgtctgcct tctttacgtt ccataggcct 420
 taagtgtttg gagcatttgt tttctttcgc cttattggag atgttccaat tgatacgttc 480
 ctgatggaga tgcttgaatc accttctgat tcataa 516

<210> 16
 <211> 528
 <212> DNA
 <213> *Amblyomma americanum*

<400> 16
 attccacatt ttgaagagct tccccttgag gaccgcatgg tggtgctcaa ggctggctgg 60
 aacgagctgc tcattgctgc tttctccac cgttctgttg acgtgcgtga tggcattgtg 120
 ctcgctacag gtcttggtg gcagcggcat agtgctcatg gggctggcgt tggggccata 180
 tttgataggg ttctcactga actggttagca aagatgcgtg agatgaagat ggaccgcaact 240
 gagcttggat gcctgcttgc tgtggtactt ttaaatcctg aggccaaggg gctgcggacc 300
 tgcccaagtg gaggccctga gggagaaagt gtatctgcct tgggaagagca ctgccggcag 360
 cagtaccag accagcctgg gcgctttgcc aagctgctgc tgcggttgcc agctctgcgc 420
 agtatggcc tcaagtgcct cgaacatctc tttttcttca agctcatcgg ggacacgccc 480
 atcgacaact ttcttcttct catgctggag gcccctctg acccctaa 528

<210> 17
 <211> 531
 <212> DNA

<213> *Amblyomma americanum*

<400> 17

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attccgcact tcgaagagct tcccatcgag gatcgccaccg cgctgctcaa agccgggtgg      60
aacgaactgc ttattgccgc cttttcgcac cgttctgtgg cggtgcgcga cggcatcggt      120
ctggccaccg ggctggtggt gcagcggcac agcgcacacg gcgcaggcgt tggcgacatc      180
ttcgaccgcg tactagccga gctggtggcc aagatgcgcg acatgaagat ggacaaaacg      240
gagctcggtt gcctgcgcgc cgtggtgctc ttcaatccag acgccaaggg tctccgaaac      300
gccaccagag tagaggcgct ccgcgagaag gtgtatgcgg cgctggagga gcaactgccgt      360
cggcaccacc cggaccaacc gggtcgcttc ggcaagctgc tgctgcggct gcctgccttg      420
cgcagcatcg ggctcaaatg cctcgagcat ctgttcttct tcaagctcat cggagacact      480
cccatagaca gcttcctgct caacatgctg gaggcaccgg cagacccta g      531

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<210> 18

<211> 552

<212> DNA

<213> *Celuca pugilator*

<400> 18

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atcccacact tcacagacct tcccatagag gaccaagtgg tattactcaa agccgggtgg      60
aacgagttgc ttattgcctc attctcacac cgtagcatgg gcgtggagga tggcatcggt      120
ctggccacag ggctcgtgat ccacagaagt agtgtcacc aggctggagt gggtgccata      180
tttgatcgtg tcctctctga gctggtggcc aagatgaagg agatgaagat tgacaagaca      240
gagctggggt gccttcgctc catcgtcctg ttcaaccacg atgccaaagg actaaactgc      300
gtcaatgatg tggagatctt gcgtgagaag gtgtatgctg ccctggagga gtacacacga      360
accacttacc ctgatgaacc tggacgcttt gccaaagtgc ttctgcgact tcctgcactc      420
aggctctatg gcctgaagtg tcttgagtac ctcttcctgt ttaagctgat tggagacact      480
cccctggaca gctacttgat gaagatgctc gtagacaacc caataacaag cgtcactccc      540
cccaccagct ag      552

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<210> 19

<211> 531

<212> DNA

<213> *Tenebrio molitor*

<400> 19

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atacctcact ttacctcggt gccgatgtcg gaccagggtgc ttttattgag ggcaggatgg      60
aatgaattgc tcatcgccgc attctcgcac agatctatac aggcgcagga tgccatcggt      120
ctagccacgg ggttgacagt taacaaaacg tcggcgcacg ccgtgggcgt gggcaacatc      180

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tacgaccgcg tcctctccga gctggtgaac aagatgaaag agatgaagat ggacaagacg 240
 gagctgggct gcttgagagc catcatcctc tacaacccca cgtgtcgcgg catcaagtcc 300
 gtgcaggaag tggagatgct gcgtgagaaa atttacggcg tgctggaaga gtacaccagg 360
 accaccacc cgaacgagcc cggcagggtc gccaaactgc ttctgcgctt cccggccctc 420
 aggtccatcg ggttgaaatg ttccgaacac ctctttttct tcaagctgat cgggtgatgtt 480
 ccaatagaca cgttcctgat ggagatgctg gagtctccgg cggacgctta g 531

<210> 20
 <211> 531
 <212> DNA
 <213> *Apis mellifera*

<400> 20
 atccccgatt ttacctcggt gccactggag gatcaggtag ttctgctcag ggccgggttg 60
 aacgagttgc tgatagcctc cttttcccac cgttccatcg acgtgaagga cggatatcgtg 120
 ctggcgacgg ggatcacctg gcatcggaac tcggcgagc aggccggcgt gggcacgata 180
 ttcgaccgtg tcctctcgga gcttgtctcg aaaatgcgtg aaatgaagat ggacaggaca 240
 gagcttggct gtctcagatc tataatactc ttcaatcccg aggttcgagg actgaaatcc 300
 atccaggaag tgaccctgct ccgtgagaag atctacggcg ccctggaggg ttattgccgc 360
 gtagcttggc ccgacgacgc tggaagattc gcgaaattac ttctacgcct gcccgccatc 420
 cgctcgatcg gattaaagtg cctcgagtag ctgttcttct tcaaaatgat cggtgacgta 480
 ccgatcgacg attttctcgt ggagatgtta gaatcgcgat cagatcctta g 531

<210> 21
 <211> 210
 <212> PRT
 <213> *Locusta migratoria*

<400> 21

His Thr Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Lys Arg Val
 1 5 10 15

Glu Cys Lys Ala Glu Asn Gln Val Glu Tyr Glu Leu Val Glu Trp Ala
 20 25 30

Lys His Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu
 35 40 45

Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His
 50 55 60

Arg Ser Val Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Leu Thr
65 70 75 80

Val His Arg Asn Ser Ala His Gln Ala Gly Val Gly Thr Ile Phe Asp
85 90 95

Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp
100 105 110

Lys Thr Glu Leu Gly Cys Leu Arg Ser Val Ile Leu Phe Asn Pro Glu
115 120 125

Val Arg Gly Leu Lys Ser Ala Gln Glu Val Glu Leu Leu Arg Glu Lys
130 135 140

Val Tyr Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asp Glu
145 150 155 160

Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser
165 170 175

Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Arg Leu Ile Gly
180 185 190

Asp Val Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ser
195 200 205

Asp Ser
210

<210> 22
<211> 228
<212> PRT
<213> Amblyomma americanum

<400> 22

Pro Pro Glu Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Leu Arg Val
1 5 10 15

Glu Ser Gln Thr Gly Thr Leu Ser Glu Ser Ala Gln Gln Gln Asp Pro
20 25 30

Val Ser Ser Ile Cys Gln Ala Ala Asp Arg Gln Leu His Gln Leu Val
35 40 45

Gln Trp Ala Lys His Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp
50 55 60

Arg Met Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala
65 70 75 80

Phe Ser His Arg Ser Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr
85 90 95

Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly Ala
100 105 110

Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met
115 120 125

Lys Met Asp Arg Thr Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe
130 135 140

Asn Pro Glu Ala Lys Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu
145 150 155 160

Gly Glu Ser Val Ser Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro
165 170 175

Asp Gln Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu
180 185 190

Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu
195 200 205

Ile Gly Asp Thr Pro Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala
210 215 220

Pro Ser Asp Pro
225

<210> 23
<211> 230
<212> PRT
<213> Amblyomma americanum

<400> 23

Ser Pro Asp Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Met Arg Val
1 5 10 15

Glu Gln Pro Ala Pro Ser Val Leu Ala Gln Thr Ala Ala Ser Gly Arg
20 25 30

Asp Pro Val Asn Ser Met Cys Gln Ala Ala Pro Pro Leu His Glu Leu
 35 40 45
 Val Gln Trp Ala Arg Arg Ile Pro His Phe Glu Glu Leu Pro Ile Glu
 50 55 60
 Asp Arg Thr Ala Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala
 65 70 75 80
 Ala Phe Ser His Arg Ser Val Ala Val Arg Asp Gly Ile Val Leu Ala
 85 90 95
 Thr Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly
 100 105 110
 Asp Ile Phe Asp Arg Val Leu Ala Glu Leu Val Ala Lys Met Arg Asp
 115 120 125
 Met Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Val Val Leu
 130 135 140
 Phe Asn Pro Asp Ala Lys Gly Leu Arg Asn Ala Thr Arg Val Glu Ala
 145 150 155 160
 Leu Arg Glu Lys Val Tyr Ala Ala Leu Glu Glu His Cys Arg Arg His
 165 170 175
 His Pro Asp Gln Pro Gly Arg Phe Gly Lys Leu Leu Leu Arg Leu Pro
 180 185 190
 Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe
 195 200 205
 Lys Leu Ile Gly Asp Thr Pro Ile Asp Ser Phe Leu Leu Asn Met Leu
 210 215 220
 Glu Ala Pro Ala Asp Pro
 225 230
 <210> 24
 <211> 266
 <212> PRT
 <213> Celuca pugilator
 <400> 24
 Ser Asp Met Pro Ile Ala Ser Ile Arg Glu Ala Glu Leu Ser Val Asp
 1 5 10 15

Pro Ile Asp Glu Gln Pro Leu Asp Gln Gly Val Arg Leu Gln Val Pro
 20 25 30

Leu Ala Pro Pro Asp Ser Glu Lys Cys Ser Phe Thr Leu Pro Phe His
 35 40 45

Pro Val Ser Glu Val Ser Cys Ala Asn Pro Leu Gln Asp Val Val Ser
 50 55 60

Asn Ile Cys Gln Ala Ala Asp Arg His Leu Val Gln Leu Val Glu Trp
 65 70 75 80

Ala Lys His Ile Pro His Phe Thr Asp Leu Pro Ile Glu Asp Gln Val
 85 90 95

Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser
 100 105 110

His Arg Ser Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu
 115 120 125

Val Ile His Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe
 130 135 140

Asp Arg Val Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile
 145 150 155 160

Asp Lys Thr Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro
 165 170 175

Asp Ala Lys Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu
 180 185 190

Lys Val Tyr Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp
 195 200 205

Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg
 210 215 220

Ser Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile
 225 230 235 240

Gly Asp Thr Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn
 245 250 255

Pro Asn Thr Ser Val Thr Pro Pro Thr Ser
260 265

<210> 25
<211> 229
<212> PRT
<213> Tenebrio molitor

<400> 25

Ala Glu Met Pro Leu Asp Arg Ile Ile Glu Ala Glu Lys Arg Ile Glu
1 5 10 15

Cys Thr Pro Ala Gly Gly Ser Gly Gly Val Gly Glu Gln His Asp Gly
20 25 30

Val Asn Asn Ile Cys Gln Ala Thr Asn Lys Gln Leu Phe Gln Leu Val
35 40 45

Gln Trp Ala Lys Leu Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp
50 55 60

Gln Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala
65 70 75 80

Phe Ser His Arg Ser Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr
85 90 95

Gly Leu Thr Val Asn Lys Thr Ser Ala His Ala Val Gly Val Gly Asn
100 105 110

Ile Tyr Asp Arg Val Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met
115 120 125

Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr
130 135 140

Asn Pro Thr Cys Arg Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu
145 150 155 160

Arg Glu Lys Ile Tyr Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His
165 170 175

Pro Asn Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala
180 185 190

Leu Arg Ser Ile Gly Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys

195 200 205
 Leu Ile Gly Asp Val Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu
 210 215 220

 Ser Pro Ala Asp Ala
 225

 <210> 26
 <211> 226
 <212> PRT
 <213> Apis mellifera

 <400> 26

 His Ser Asp Met Pro Ile Glu Arg Ile Leu Glu Ala Glu Lys Arg Val
 1 5 10 15

 Glu Cys Lys Met Glu Gln Gln Gly Asn Tyr Glu Asn Ala Val Ser His
 20 25 30

 Ile Cys Asn Ala Thr Asn Lys Gln Leu Phe Gln Leu Val Ala Trp Ala
 35 40 45

 Lys His Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu
 50 55 60

 Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His
 65 70 75 80

 Arg Ser Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr
 85 90 95

 Val His Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp
 100 105 110

 Arg Val Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp
 115 120 125

 Arg Thr Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu
 130 135 140

 Val Arg Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys
 145 150 155 160

 Ile Tyr Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp
 165 170 175

Ala Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser
 180 185 190

Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly
 195 200 205

Asp Val Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser
 210 215 220

Asp Pro
 225

<210> 27
 <211> 176
 <212> PRT
 <213> Locusta migratoria

<400> 27

Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
 20 25 30

Val Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Leu Thr Val His
 35 40 45

Arg Asn Ser Ala His Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val
 50 55 60

Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Lys Thr
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Val Ile Leu Phe Asn Pro Glu Val Arg
 85 90 95

Gly Leu Lys Ser Ala Gln Glu Val Glu Leu Leu Arg Glu Lys Val Tyr
 100 105 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asp Glu Pro Gly
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser Ile Gly
 130 135 140

Leu Lys Cys Leu Glu His Leu Phe Phe Phe Arg Leu Ile Gly Asp Val

145 150 155 160
 Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ser Asp Ser
 165 170 175

 <210> 28
 <211> 175
 <212> PRT
 <213> Amblyomma americanum

 <400> 28

 Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp Arg Met Val Leu Leu
 1 5 10 15

 Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
 20 25 30

 Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr Gly Leu Val Val Gln
 35 40 45

 Arg His Ser Ala His Gly Ala Gly Val Gly Ala Ile Phe Asp Arg Val
 50 55 60

 Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Arg Thr
 65 70 75 80

 Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe Asn Pro Glu Ala Lys
 85 90 95

 Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu Gly Glu Ser Val Ser
 100 105 110

 Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro Asp Gln Pro Gly Arg
 115 120 125

 Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu
 130 135 140

 Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro
 145 150 155 160

 Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala Pro Ser Asp Pro
 165 170 175

 <210> 29
 <211> 176
 <212> PRT

<213> Amblyomma americanum

<400> 29

Ile	Pro	His	Phe	Glu	Glu	Leu	Pro	Ile	Glu	Asp	Arg	Thr	Ala	Leu	Leu
1				5					10					15	

Lys	Ala	Gly	Trp	Asn	Glu	Leu	Leu	Ile	Ala	Ala	Phe	Ser	His	Arg	Ser
		20						25					30		

Val	Ala	Val	Arg	Asp	Gly	Ile	Val	Leu	Ala	Thr	Gly	Leu	Val	Val	Gln
	35						40					45			

Arg	His	Ser	Ala	His	Gly	Ala	Gly	Val	Gly	Asp	Ile	Phe	Asp	Arg	Val
	50					55					60				

Leu	Ala	Glu	Leu	Val	Ala	Lys	Met	Arg	Asp	Met	Lys	Met	Asp	Lys	Thr
65					70					75					80

Glu	Leu	Gly	Cys	Leu	Arg	Ala	Val	Val	Leu	Phe	Asn	Pro	Asp	Ala	Lys
			85						90					95	

Gly	Leu	Arg	Asn	Ala	Thr	Arg	Val	Glu	Ala	Leu	Arg	Glu	Lys	Val	Tyr
			100					105					110		

Ala	Ala	Leu	Glu	Glu	His	Cys	Arg	Arg	His	His	Pro	Asp	Gln	Pro	Gly
		115					120					125			

Arg	Phe	Gly	Lys	Leu	Leu	Leu	Arg	Leu	Pro	Ala	Leu	Arg	Ser	Ile	Gly
	130					135					140				

Leu	Lys	Cys	Leu	Glu	His	Leu	Phe	Phe	Phe	Lys	Leu	Ile	Gly	Asp	Thr
145					150					155					160

Pro	Ile	Asp	Ser	Phe	Leu	Leu	Asn	Met	Leu	Glu	Ala	Pro	Ala	Asp	Pro
				165					170					175	

<210> 30

<211> 183

<212> PRT

<213> Celuca pugilator

<400> 30

Ile	Pro	His	Phe	Thr	Asp	Leu	Pro	Ile	Glu	Asp	Gln	Val	Val	Leu	Leu
1				5					10					15	

Lys	Ala	Gly	Trp	Asn	Glu	Leu	Leu	Ile	Ala	Ser	Phe	Ser	His	Arg	Ser
		20						25					30		

Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu Val Ile His
 35 40 45

Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe Asp Arg Val
 50 55 60

Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile Asp Lys Thr
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro Asp Ala Lys
 85 90 95

Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu Lys Val Tyr
 100 105 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp Glu Pro Gly
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly
 130 135 140

Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile Gly Asp Thr
 145 150 155 160

Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn Pro Asn Thr
 165 170 175

Ser Val Thr Pro Pro Thr Ser
 180

<210> 31
 <211> 176
 <212> PRT
 <213> Tenebrio molitor

<400> 31

Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp Gln Val Leu Leu Leu
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
 20 25 30

Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr Gly Leu Thr Val Asn
 35 40 45

Lys Thr Ser Ala His Ala Val Gly Val Gly Asn Ile Tyr Asp Arg Val
 50 55 60

Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met Lys Met Asp Lys Thr
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr Asn Pro Thr Cys Arg
 85 90 95

Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu Arg Glu Lys Ile Tyr
 100 105 110

Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asn Glu Pro Gly
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly
 130 135 140

Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Val
 145 150 155 160

Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ala Asp Ala
 165 170 175

<210> 32
 <211> 176
 <212> PRT
 <213> Apis mellifera

<400> 32

Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu
 1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser
 20 25 30

Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr Val His
 35 40 45

Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val
 50 55 60

Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp Arg Thr
 65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu Val Arg
 85 90 95

Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys Ile Tyr
 100 105 110

Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp Ala Gly
 115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser Ile Gly
 130 135 140

Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly Asp Val
 145 150 155 160

Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser Asp Pro
 165 170 175

<210> 33
 <211> 441
 <212> DNA
 <213> *Saccharomyces cerevisiae*

<400> 33
 atgaagctac tgtcttctat cgaacaagca tgcgatattt gccgacttaa aaagctcaag 60
 tgctccaaag aaaaaccgaa gtgcgccaag tgtctgaaga acaactggga gtgtcgctac 120
 tctcccaaaa ccaaagggtc tccgctgact agggcacatc tgacagaagt ggaatcaagg 180
 ctagaaagac tggaacagct atttctactg atttttcctc gagaagacct tgacatgatt 240
 ttgaaaatgg attctttaca ggatataaaa gcattgttaa caggattatt tgtacaagat 300
 aatgtgaata aagatgccgt cacagataga ttggcttcag tggagactga tatgcctcta 360
 acattgagac agcatagaat aagtgcgaca tcatcatcgg aagagagtag taacaaagggt 420
 caaagacagt tgactgtatc g 441

<210> 34
 <211> 147
 <212> PRT
 <213> *Saccharomyces cerevisiae*

<400> 34
 Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
 1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
 20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro

35	40	45
Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu		
50	55	60
Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile		
65	70	75
Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu		
85	90	95
Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala		
100	105	110
Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser		
115	120	125
Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu		
130	135	140
Thr Val Ser		
145		

<210> 35
 <211> 606
 <212> DNA
 <213> Escherichia coli

<400> 35
 atgaaagcgt taacggccag gcaacaagag gtgtttgatc tcatccgtga tcacatcagc 60
 cagacaggta tgccgccgac gcgtgcggaa atcgcgcagc gtttgggggtt ccgttcccca 120
 aacgcggctg aagaacatct gaaggcgctg gcacgcaaag gcgttattga aattgtttcc 180
 ggcgcatcac gcgggattcg tctgttgacg gaagaggaag aagggttgcc gctggtaggt 240
 cgtgtggctg ccggtgaacc acttctggcg caacagcata ttgaagggtca ttatcaggtc 300
 gatccttctt tattcaagcc gaatgctgat ttctgctgc gcgtcagcgg gatgtcgatg 360
 aaagatatcg gcattatgga tgggtgacttg ctggcagtgc ataaaactca ggatgtacgt 420
 aacggtcagg tcgttgctgc acgtattgat gacgaagtta ccgttaagcg cctgaaaaaa 480
 cagggcaata aagtcgaact gttgccagaa aatagcgagt ttaaaccaat tgctgtagat 540
 cttcgtcagc agagcttcac cattgaaggg ctggcggttg gggttattcg caacggcgac 600
 tggctg 606

<210> 36

<211> 202

<212> PRT

<213> Escherichia coli

<400> 36

Met Lys Ala Leu Thr Ala Arg Gln Gln Glu Val Phe Asp Leu Ile Arg
 1 5 10 15

Asp His Ile Ser Gln Thr Gly Met Pro Pro Thr Arg Ala Glu Ile Ala
 20 25 30

Gln Arg Leu Gly Phe Arg Ser Pro Asn Ala Ala Glu Glu His Leu Lys
 35 40 45

Ala Leu Ala Arg Lys Gly Val Ile Glu Ile Val Ser Gly Ala Ser Arg
 50 55 60

Gly Ile Arg Leu Leu Gln Glu Glu Glu Glu Gly Leu Pro Leu Val Gly
 65 70 75 80

Arg Val Ala Ala Gly Glu Pro Leu Leu Ala Gln Gln His Ile Glu Gly
 85 90 95

His Tyr Gln Val Asp Pro Ser Leu Phe Lys Pro Asn Ala Asp Phe Leu
 100 105 110

Leu Arg Val Ser Gly Met Ser Met Lys Asp Ile Gly Ile Met Asp Gly
 115 120 125

Asp Leu Leu Ala Val His Lys Thr Gln Asp Val Arg Asn Gly Gln Val
 130 135 140

Val Val Ala Arg Ile Asp Asp Glu Val Thr Val Lys Arg Leu Lys Lys
 145 150 155 160

Gln Gly Asn Lys Val Glu Leu Leu Pro Glu Asn Ser Glu Phe Lys Pro
 165 170 175

Ile Val Val Asp Leu Arg Gln Gln Ser Phe Thr Ile Glu Gly Leu Ala
 180 185 190

Val Gly Val Ile Arg Asn Gly Asp Trp Leu
 195 200

<210> 37

<211> 271

<212> DNA

<213> herpes simplex virus 7

<400> 37

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atggggcccta aaaagaagcg taaagtcgcc ccccgaccg atgtcagcct gggggacgag      60
ctccacttag acggcgagga cgtggcgatg gcgcatgccg acgcgctaga cgatttcgat      120
ctggacatgt tgggggacgg ggattccccg gggccgggat ttacccccca cgactccgcc      180
ccctacggcg ctctggatat ggccgacttc gagtttgagc agatgtttac cgatgccctt      240
ggaattgacg agtacggtgg ggaattcccg g                                271
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<210> 38

<211> 90

<212> PRT

<213> herpes simplex virus 7

<400> 38

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Met Gly Pro Lys Lys Lys Arg Lys Val Ala Pro Pro Thr Asp Val Ser
1                               5                               10                               15
```

```
Leu Gly Asp Glu Leu His Leu Asp Gly Glu Asp Val Ala Met Ala His
20                               25                               30
```

```
Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Asp Gly Asp
35                               40                               45
```

```
Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro Tyr Gly Ala
50                               55                               60
```

```
Leu Asp Met Ala Asp Phe Glu Phe Glu Gln Met Phe Thr Asp Ala Leu
65                               70                               75                               80
```

```
Gly Ile Asp Glu Tyr Gly Gly Glu Phe Pro
85                               90
```

<210> 39

<211> 307

<212> DNA

<213> Saccharomyces cerevisiae

<400> 39

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atgggtgctc ctccaaaaaa gaagagaaag gtagctggtg tcaataaaga tatcgaggag      60
tgcaatgccg tcattgagca gtttatcgac tacctgcgca ccggacagga gatgccgatg      120
gaaatggcgg atcaggcgat taacgtggtg cggggcatga cgccgaaaac cattcttcac      180
gccggggccgc cgatccagcc tgactggctg aaatcgaatg gttttcatga aattgaagcg      240
gatgttaacg ataccagcct cttgctgagt ggagatgcct cctaccctta tgatgtgccg      300
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gattatg

307

<210> 40
 <211> 102
 <212> PRT
 <213> *Saccharomyces cerevisiae*

<400> 40

Met Gly Ala Pro Pro Lys Lys Lys Arg Lys Val Ala Gly Ile Asn Lys
 1 5 10 15

Asp Ile Glu Glu Cys Asn Ala Ile Ile Glu Gln Phe Ile Asp Tyr Leu
 20 25 30

Arg Thr Gly Gln Glu Met Pro Met Glu Met Ala Asp Gln Ala Ile Asn
 35 40 45

Val Val Pro Gly Met Thr Pro Lys Thr Ile Leu His Ala Gly Pro Pro
 50 55 60

Ile Gln Pro Asp Trp Leu Lys Ser Asn Gly Phe His Glu Ile Glu Ala
 65 70 75 80

Asp Val Asn Asp Thr Ser Leu Leu Leu Ser Gly Asp Ala Ser Tyr Pro
 85 90 95

Tyr Asp Val Pro Asp Tyr
 100

<210> 41
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> GAL4 response element

<400> 41

ggagtactgt cctccgagc

19

<210> 42
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2xLexAop response element

<400> 42

ctgctgtata taaaaccagt gggttatatgt acagta

36

<210> 43
 <211> 334
 <212> PRT
 <213> Choristoneura fumiferana

<400> 43

Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys Arg Lys Glu
 1 5 10 15

Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr
 20 25 30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro
 35 40 45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys
 50 55 60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn
 65 70 75 80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu
 85 90 95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln
 100 105 110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr
 115 120 125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly
 130 135 140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu
 145 150 155 160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr
 165 170 175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr
 180 185 190

Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu
 195 200 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His

210 215 220
 Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu
 225 230 235 240
 Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr
 245 250 255
 Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser
 260 265 270
 Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu
 275 280 285
 Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg
 290 295 300
 Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser
 305 310 315 320
 His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn Leu
 325 330
 <210> 44
 <211> 549
 <212> PRT
 <213> Drosophila melanogaster
 <400> 44
 Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg
 1 5 10 15
 Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser
 20 25 30
 Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp
 35 40 45
 Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln
 50 55 60
 His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln
 65 70 75 80
 Ala Arg Asn Ile Pro Ser Leu Thr Tyr Asn Gln Leu Ala Val Ile Tyr
 85 90 95

Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp
 100 105 110

Leu Arg Arg Ile Met Ser Gln Pro Asp Glu Asn Glu Ser Gln Thr Asp
 115 120 125

Val Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu
 130 135 140

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln
 145 150 155 160

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met
 165 170 175

Leu Arg Met Ala Arg Arg Tyr Asp His Ser Ser Asp Ser Ile Phe Phe
 180 185 190

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met
 195 200 205

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Phe Ser
 210 215 220

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile
 225 230 235 240

Phe Ser Asp Arg Pro Gly Leu Glu Lys Ala Gln Leu Val Glu Ala Ile
 245 250 255

Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His
 260 265 270

Cys Gly Asp Ser Met Ser Leu Val Phe Tyr Ala Lys Leu Leu Ser Ile
 275 280 285

Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe
 290 295 300

Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile
 305 310 315 320

Trp Asp Val His Ala Ile Pro Pro Ser Val Gln Ser His Leu Gln Ile
 325 330 335

Thr Gln Glu Glu Asn Glu Arg Leu Glu Arg Ala Glu Arg Met Arg Ala
 340 345 350

Ser Val Gly Gly Ala Ile Thr Ala Gly Ile Asp Cys Asp Ser Ala Ser
 355 360 365

Thr Ser Ala Ala Ala Ala Ala Ala Gln His Gln Pro Gln Pro Gln Pro
 370 375 380

Gln Pro Gln Pro Ser Ser Leu Thr Gln Asn Asp Ser Gln His Gln Thr
 385 390 395 400

Gln Pro Gln Leu Gln Pro Gln Leu Pro Pro Gln Leu Gln Gly Gln Leu
 405 410 415

Gln Pro Gln Leu Gln Pro Gln Leu Gln Thr Gln Leu Gln Pro Gln Ile
 420 425 430

Gln Pro Gln Pro Gln Leu Leu Pro Val Ser Ala Pro Val Pro Ala Ser
 435 440 445

Val Thr Ala Pro Gly Ser Leu Ser Ala Val Ser Thr Ser Ser Glu Tyr
 450 455 460

Met Gly Gly Ser Ala Ala Ile Gly Pro Ile Thr Pro Ala Thr Thr Ser
 465 470 475 480

Ser Ile Thr Ala Ala Val Thr Ala Ser Ser Thr Thr Ser Ala Val Pro
 485 490 495

Met Gly Asn Gly Val Gly Val Gly Val Gly Val Gly Gly Asn Val Ser
 500 505 510

Met Tyr Ala Asn Ala Gln Thr Ala Met Ala Leu Met Gly Val Ala Leu
 515 520 525

His Ser His Gln Glu Gln Leu Ile Gly Gly Val Ala Val Lys Ser Glu
 530 535 540

His Ser Thr Thr Ala
 545

<210> 45
 <211> 1288
 <212> DNA
 <213> *Choristoneura fumiferana*

<400> 45
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accaaaaaatg cggtttatat ttgtaaattc ggtcacgctt gcgaaatgga catgtacatg 180
cgacggaaaat gccaggagtg ccgcctgaag aagtgccttag ctgtaggcat gaggcctgag 240
tgcgtagtac ccgagactca gtgcgccatg aagcggaaaag agaagaaagc acagaaggag 300
aaggacaaac tgctgtcag cacgacgacg gtggacgacc acatgccgcc cattatgcag 360
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gatgccgcgt ccggccgcgc tgctctga 1288

<210> 46
<211> 309
<212> DNA
<213> Simian virus 40

<400> 46
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tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccata ccgcccctaa 180
ctccgcccag ttccgcccata tctccgcccc atggctgact aatTTTTTTTT atttatgcag 240
aggccgaggc cgctcggcc tctgagctat tccagaagta gtgaggaggc ttttttggag 300

gcctaggct 309

<210> 47
 <211> 789
 <212> DNA
 <213> Mus musculus

<400> 47
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 ctacggggcag gctggaacga gctgctgatc gcctccttct cccacogctc catagctgtg 360
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 aaggggctct caaacctgc tgagggtggag gcgttgaggg agaaggtgta tgcgtcacta 600
 gaagcgtact gcaaacacaa gtaccctgag cagccgggca ggtttgccaa gctgctgctc 660
 cgctgcctg cactgcgttc catcgggctc aagtgcctgg agcacctgtt cttcttcaag 720
 ctcatcgggg acacgcccat cgacaccttc ctcatggaga tgctggaggc accacatcaa 780
 gccacctag 789

<210> 48
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic E1b minimal promoter

<400> 48
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<210> 49
 <211> 1653
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> luciferase gene

<400> 49
 atggaagacg ccaaaaacat aaagaaaggc ccggcgccat tctatcctct agaggatgga 60

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accgctggag agcaactgca taaggctatg aagagatacg ccctgggttcc tggaacaatt      120
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gttcgggttg cagaagctat gaaacgatat gggctgaata caaatcacag aatcgtcgta      240
tgcagtgaaa actctcttca attctttatg ccggtgttgg gcgcgttatt tatcggagtt      300
gcagttgctc ccgcgaacga catttataat gaacgtgaat tgctcaacag tatgaacatt      360
tcgcagccta ccgtagtgtt tgtttccaaa aaggggttgc aaaaaatttt gaacgtgcaa      420
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gcggtcggta aagttgttcc attttttgaa gcgaagggtg tggatctgga taccgggaaa     1140
acgctgggcg ttaatcagag aggcgaatta tgtgtcagag gacctatgat tatgtccggt     1200
tatgtaaaca atccggaagc gaccaacgcc ttgattgaca aggatggatg gctacattct     1260
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cccgccgccg ttgttggttt ggagcacgga aagacgatga cggaaaaaga gatcgtggat     1500
tacgtcgcca gtcaagtaac aaccgcgaaa aagttgcgcg gaggagttgt gtttgtggac     1560
gaagtaccga aaggtcttac cggaaaactc gacgcaagaa aaatcagaga gatcctcata     1620
aaggccaaga agggcggaaa gtccaaattg taa                                     1653

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<210> 50
<211> 714
<212> DNA
<213> Mus musculus

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<400> 50
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accaacatct gtcaagcagc agacaagcag ctcttcactc ttgtggagtg ggccaagagg 180
atcccacact tttctgagct gcccctagac gaccaggtca tcttgctacg ggcaggctgg 240
aacgagctgc tgatcgccctc cttctccac cgctccatag ctgtgaaaga tgggattctc 300
ctggccaccg gcctgcacgt acaccggaac agcgctcaca gtgctggggg gggcgccatc 360
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gagctgggct gcctgcgagc cattgtcctg ttcaaccctg actctaagg gctctcaaac 480
cctgctgagg tggaggcggt gagggagaag gtgtatgctg cactagaagc gtactgcaaa 540
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cgttccatcg ggctcaagtg cctggagcac ctgttcttct tcaagctcat cggggacacg 660
cccatcgaca ccttcctcat ggagatgctg gaggcaccac atcaagccac ctag 714

<210> 51
<211> 867
<212> DNA
<213> *Choristoneura fumiferana*

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tcttttggtg cagatcccag cgaggagttc cagttcctcc gcgtggggcc tgacagcaac 180
gtgcctccac gttaccgcgc gcccgctctcc tccctctgcc aaataggcaa caagcaaata 240
gcggcgttgg tggtatgggc gcgcgacatc cctcatttcg ggcagctgga gctggacgat 300
caagtggtag tcatcaaggc ctcttggaat gagctgctac tcttcgccat cgccctggcgc 360
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cgcatggacc aggccgagta cgtcgcgctc aaagccatcg tgctgctcaa ccctgatgtg 600
aaaggactga agaatcgga agaagttgac gttttgagag aaaaaatgtt ctcttgccctg 660
gacgactact gccggcggtc gcgaagcaac gaggaaggcc ggtttgctc cttgctgctg 720
cggctgccag ctctccgctc catctcgctc aagagcttcg aacacctcta cttctccac 780
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ccgatcgacg tcaatgccat gatgtaa 867

<210> 52
 <211> 711
 <212> DNA
 <213> *Locusta migratoria*

<400> 52
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 cgagtggagt gcaaagcaga aaaccaagtg gaatatgagc tgggtggagtg ggctaaacac 180
 atcccgact tcacatccct acctctggag gaccaggttc tcctcctcag agcagggttg 240
 aatgaactgc taattgcagc attttcacat cgatctgtag atgttaaaga tggcatagta 300
 cttgccactg gtctcacagt gcacgaaaat tctgcccata aagctggagt cggcacaata 360
 tttgacagag ttttgacaga actggtagca aagatgagag aaatgaaaat ggataaaaact 420
 gaacttggct gcttgcgatc tgttattctt ttcaatccag aggtgagggg tttgaaatcc 480
 gccaggaag ttgaacttct acgtgaaaaa gtatatgccg ctttggaga atatactaga 540
 acaacacatc ccgatgaacc aggaagatct gcaaaacttt tgcttcgtct gccttcttta 600
 cgttccatag gccttaagtg tttggagcat ttgtttttct ttcgccttat tggagatgtt 660
 ccaattgata cgttcctgat ggagatgctt gaatcacctt ctgattcata a 711

<210> 53
 <211> 1054
 <212> DNA
 <213> *Choristoneura fumiferana*

<400> 53
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 aaggagaagg acaaactgcc tgtcagcacg acgacggtgg acgaccacat gccgcccatt 120
 atgcagtgtg aacctccacc tcctgaagca gcaaggattc acgaagtggc cccaaggttt 180
 ctctccgaca agctggttga gacaaaccgg cagaaaaaca tccccagtt gacagccaac 240
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 gaagatttga agaggattac gcagacgtgg cagcaagcgg acgatgaaaa cgaagagtct 360
 gacactccct tccgccagat cacagagatg actatcctca cggccaact tatcgtggag 420
 ttgcggaagg gattgccagg gttcgccaaag atctcgagc ctgatcaaat tacgctgctt 480
 aaggcttgct caagtgaggt aatgatgctc cgagtcgagc gacgatacga tgcggcctca 540
 gacagtgttc tgttcgcaa caaccaagcg tacactcgcg acaactaccg caaggctggc 600
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gacaacatcc attacgcgct gctcacggct gtcgtcatct tttctgaccg gccagggttg 720
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 ctcaagaaca gaaagctgcc gcctttcttc gaggagatct gggatgtggc ggacatgtcg 960
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<210> 54

<211> 1542

<212> DNA

<213> Choristoneura fumiferana

<400> 54

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 aagaagggcc ctgcgccccg tcagcaagag gaactgtgtc tggatatgcg ggacagagcc 360
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 gagaaggaca aactgcctgt cagcacgacg acggtggacg accacatgcc gccattatg 660
 cagtgtgaac ctccacctcc tgaagcagca aggattcacg aagtgggtccc aaggtttctc 720
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aacatccatt acgcgctgct cacggctgtc gtcattcttt ctgaccggcc agggttggag 1260
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 aagaacagaa agctgccgcc ttctctcgag gagatctggg atgtggcgga catgtcgcac 1500
 acccaaccgc cgcctatcct cgagtcccc acgaatctct ag 1542

<210> 55
 <211> 1110
 <212> DNA
 <213> Choristoneura fumiferana

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<210> 56
 <211> 798
 <212> DNA

<213> *Choristoneura fumiferana*

<400> 56

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atgtgtctca tgcctggcat gacgttgac cgcaactcgg cgcagcaggc gggcggtgggc	420
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aagaatcggc aagaagttga cgttttgcca gaaaaaatgt tctcttgctt ggacgactac	600
tgccggcggt cgcgaagcaa cgaggaaggc cggtttgctt ccttgctgct ggggctgcca	660
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gaaggctcca tcagcggata catacgagag gcgctccgaa accacgcgcc tccgatcgac	780
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<210> 57

<211> 1586

<212> DNA

<213> *Bamecia argentifoli*

<400> 57

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acccttatag tcccaccaat ggaagaatag ggaaagaaga gctttcgccg gcgaatagtc	180
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accagtgtaa atatggaaat aattgtgaaa ttgacatgta catgaggcga aaatgccaaag	420
agtgtcgtct caagaagtgt ctcagcgttg gcatgaggcc agaattgtgta gttcccgaat	480
tccagtgtgc tgtgaagcga aaagagaaaa aagcgcaaaa ggacaaagat aaacctaact	540
caacgacgag ttgttctcca gatggaatca aacaagagat agatcctcaa aggctggata	600
cagattcgca gctattgtct gtaaattggag ttaaaccat tactccagag caagaagagc	660
tcatccatag gctagtttat tttaaaaatg aatatgaaca tccatcccca gaggatatca	720

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aaaggatagt taatgctgca ccagaagaag aaaatgtagc tgaagaaagg tttaggcata      780
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catatgcaac aaccattttt gctaagttac tatctgtttt aactgaacta cgaacattag     1260
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ttcatatttg atttatctca gcagggtggct cagtacttat cctcacatta ctgagctcac     1440
ggtagctca tacaattata acttgtaata tcatatcggt gatgacaaat ttgttacaat     1500
attctttggt accttaacac aatgttgatc tcataatgat gtatgaattt ttctgttttt     1560
gcaaaaaaaaa aagcggccgc gaattc                                           1586

```

<210> 58

<211> 1109

<212> DNA

<213> *Nephotetix cincticeps*

<400> 58

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tccaaatacg gcaccaattg tgaaatagac atgtatatgc ggcgcaagtg ccaggagtgc     180
cgactcaaga agtgcctcag tgtagggatg aggccagaat gtgtagtacc tgagtatcaa     240
tgtgccgtaa aaaggaaaga gaaaaaagct caaaaggaca aagataaacc tgtctcttca     300
accaatggct cgctgaaat gagaatagac caggacaacc gttgtgtggg gttgcagagt     360
gaagacaaca ggtacaactc gagtacgccc agtttcggag tcaaaccctt cagtccagaa     420
caagaggagc tcatccacag gctcgtctac ttccagaacg agtacgaaca cctgcccag     480
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tcgagcgagg tgatgatgct gcggatggcg cggaggtacg acgtccagac agactcgatc     720

```



```

ctgttcgcca acaaccagcc gtacacgcga gagtcgtaca cgatggcagg cgtgggggaa      780
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gagtatgctc tgctcacggc catcgtcata ttctccgagc ggccgaacct ggcggaagga      900
tggaagggtg agaagatcca ggagatctac ctggaggcgc tcaagtccta cgtggacaac      960
cgagtgaaac ctgcgagtcc gaccatcttc gccaaactgc tctccgttct caccgagctg     1020
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```

<210> 59

<211> 401

<212> PRT

<213> Choristoneura fumiferana

<400> 59

```

Cys Leu Val Cys Gly Asp Arg Ala Ser Gly Tyr His Tyr Asn Ala Leu
1           5           10           15

```

```

Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Val Thr Lys Asn
          20           25           30

```

```

Ala Val Tyr Ile Cys Lys Phe Gly His Ala Cys Glu Met Asp Met Tyr
          35           40           45

```

```

Met Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val
          50           55           60

```

```

Gly Met Arg Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys
65           70           75           80

```

```

Arg Lys Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser
          85           90           95

```

```

Thr Thr Thr Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro
          100          105          110

```

```

Pro Pro Pro Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu
          115          120          125

```

```

Ser Asp Lys Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu
          130          135          140

```

```

Thr Ala Asn Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp
          145          150          155          160

```

Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr
 165 170 175
 Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg
 180 185 190
 Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe
 195 200 205
 Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile
 210 215 220
 Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala
 225 230 235 240
 Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln
 245 250 255
 Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile
 260 265 270
 Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp
 275 280 285
 Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg
 290 295 300
 Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr
 305 310 315 320
 Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala
 325 330 335
 Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu
 340 345 350
 Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu
 355 360 365
 Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala
 370 375 380
 Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn
 385 390 395 400

Leu

<210> 60

<211> 825

<212> DNA

<213> *Drosophila melanogaster*

<400> 60

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tacaaggggtg ccgtgtcggc cctgtgccaa gtggtcaaca aacagctctt ccagatggtc      180
gaatacgcgc gcatgatgcc gcactttgcc caggtgccgc tggacgacca ggtgattctg      240
ctgaaagccg cttggatcga gctgctcatt gcgaacgtgg cctgggtgcag catcgtttctg      300
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cgacgatcac cgggccttca gccccagcag ctgttcctca accagagctt ctcgtaecat      420
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agtgtaaaga tgaagcggct gaatctcgac cgacgcgagc tgtcctgctt gaaggccatc      540
atactgtaca acccggacat acgcgggcatc aagagccggg cggagatcga gatgtgccgc      600
gagaaggtgt acgcttgctt ggacgagcac tgccgcctgg aacatccggg cgacgatgga      660
cgctttgcgc aactgctgct gogtctgccc gctttgcgat cgatcagcct gaagtgccag      720
gatcacctgt tcctcttccg cattaccagc gaccggccgc tggaggagct ctttctcgag      780
cagctggagg cgccgccgcc acccggcctg gcgatgaaac tggag                          825

```